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Manuscripts

The Editors of Diabetes: *The Journal of the American Diabetes Association* are grateful to the following persons who, although not members of the Editorial Board during the year 1961, evaluated scientific contributions to the Journal.

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Memoranda

Insulin Resistance with High Levels of Circulating Insulin-like Activity Demonstrable in Vitro and in Vivo

J. C. Shipp, M.D., R. O. Russell, M.D., J. Steinke, M.D., M. L. Mitchell, M.D., and W. B. Hadley, M.D., Boston

The purpose of the present study was to examine some of the possible sites of interference with insulin action during insulin resistance. In theory, this interference can result from binding or inactivation of insulin in the vascular or interstitial compartments, from altered transfer of insulin across the capillary membrane to the reactive cell site or from a failure of the cell to respond normally to insulin (figure 1).

This report includes studies over a six-month period in a patient with insulin resistance and deals primarily with circulating factors which may alter the action of insulin. An increased serum binding capacity and precipitin antibodies were shown. High levels of circulating insulin-like activity were demonstrated by in vitro and in vivo methods. These abnormalities were no longer present when insulin sensitivity returned following prednisone therapy.

CASE REPORT

E.P., Joslin Clinic No. 54010, a seventy-three-year-old white female, was admitted to the New England Deaconess Hospital for the third time on Sept. 3, 1959, with diabetic acidosis.

The major clinical features are shown in table 1. Diabetes mellitus was discovered at age fifty-nine and treated with diet alone. A sister and paternal grandfather were diabetic. Thirteen years later, at age seventy-two, she was placed on 75 units of Protamine Zinc Insulin daily following a foot infection. She was first seen at the Joslin Clinic in May of 1959 when she was admitted to the New England Deaconess Hospital in diabetic coma precipitated by a foot infection. The keto-acidosis cleared with 1,200 units of insulin; subsequently 400 to 600 units of insulin daily were required to prevent ketosis. In July she was hospitalized because of bilateral foot ulcers. At this time her serum showed high levels of insulin-like activity and increased insulin binding capacity (see table 1). Tolbutamide (2.0 gm. daily for two days), tolbutamide (2.0 gm.) plus dexamethasone (3.0 mg.) daily for two days and dexamethasone (1.5 mg. for one day) alone had no effect on the insulin requirement. She was discharged on 650 units of U-500 insulin plus 100 units of Crystalline Insulin daily. Over the next four weeks the insulin need rose to 1,250 units twice daily. The detailed sequence of insulin requirements is shown in figure 2.

One day before the third hospital admission on Sept. 3, 1959, a necrotic area was noted on the right thigh at the site of the insulin injection. During the three hours before admission nausea and vomiting began. When first seen she was semi-stuporous with a blood pressure of 150/40 mm. of mercury, a pulse of 96 per minute and respirations of 36 per minute which were Kussmaul in type. There was an acetone odor to her breath. She had diabetic retinopathy, warm dry skin and a 0.5 cm. necrotic area on the right thigh. Heart and lungs were normal. Moderate hepatosplenomegaly was noted. Blood sugar was 726 mg. per 100 ml., serum acetone was positive in a 1:8 dilution; serum sodium, potassium and chloride were 126, 3.5, and 94 mEq./L., respectively. Arterial carbon dioxide content was 5 mM/L., arterial *p*H 7.22 with a calculated pCO_2 of 12 mm. of mercury. Hemoglobin was 13.0 gm. per cent. There were 8,400 leukocytes per cu. mm.; the differential smear showed 4 per cent eosinophils.

Over a period of twenty-four hours the patient received 6,000 units of insulin. Of this amount 2,000 units of U-5,000

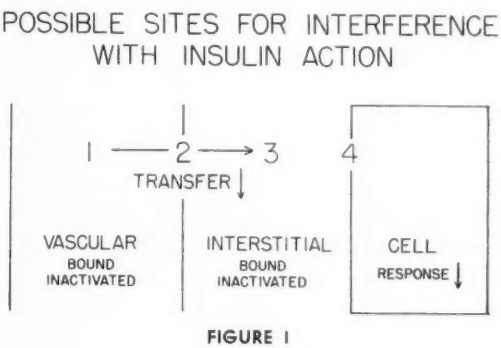

FIGURE 1

Presented at the Twentieth Annual Meeting of the American Diabetes Association in Miami Beach on June 12, 1960.

From the Joslin Clinic and New England Deaconess Hospital, Department of Medicine, Harvard Medical School and Lemuel Shattuck Hospital, Boston, Massachusetts. Dr. Shipp is a Fellow of Medical Foundation of Boston. Present address: University of Florida School of Medicine, Gainesville, Florida. Dr. Steinke is the recipient of a Research Fellowship of the American Diabetes Association.

TABLE 1

Insulin resistance (J.C. No. 54010)

Age	Date	Clinical	Insulin (U./day)	Anti-bodies	Binding	ILA* In vitro (mu/ml.)	In vivo
59	1945	Onset	0				
72	1958	Foot inf.	75				
73	1959						
	May	Coma (1,200 U.)	600				
	July	Foot inf.	600		10†	13	
	Aug.		2,500				
	Sept.	Coma (9,000 U.)	1,200	+	>20†	175	++++
	Oct.	Prednisone	1,200				
	Dec.		50		†	2.1	

* Insulin-like activity. † Level in insulin treated diabetic.

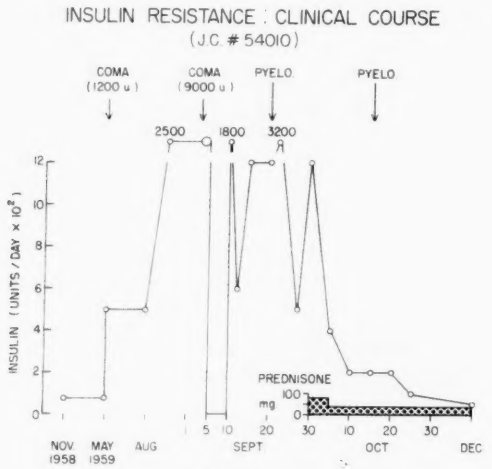

FIGURE 2

insulin‡ were given intravenously over about thirty seconds. Within five minutes she developed a rapid jerky motion of the jaw and became unresponsive. Blood pressure was unobtainable. Profuse sweating and an urticarial rash appeared. She responded to 100 mg. of hydrocortisone plus 40 mg. of metaraminol, given slowly intravenously, with a rise in blood pressure to 90/60. This was thought to be an anaphylactic reaction to intravenous insulin. Blood sugar thirty minutes after this episode was 330 mg. per 100 ml. Urine output, which had averaged 75 ml. per hour, fell to less than 15 ml. During the second day the blood sugar was 400 to 500 mg. per 100 ml. and she was given 3,000 units of U-5,000 insulin subcutaneously, and 100 mg. of hydrocortisone to help maintain blood pressure.

For the next five days urine output remained low at between 300 and 600 ml. daily. During this time, after the initial 9,000 units of insulin, she was given no insulin but required frequent intravenous and oral glucose to prevent symptomatic hypoglycemia. Blood glucose remained between 30 and 80 mg. per 100 ml. On the sixth day, urine output increased from 15 to 100 ml. per hour, blood sugar rose to 470 mg. per 100 ml. and she again required insulin.

‡ Courtesy of Dr. William Kirtley of Eli Lilly and Company, Indianapolis.

Insulin dosage rose steadily seeming to stabilize at around 1,200 units daily. With a urinary tract infection she required 3,200 units daily for three days. As the infection was controlled with chloramphenicol, insulin dose returned to around 1,200 units. Throughout this period insulin was given in amounts adequate to prevent ketosis. Blood sugars varied between 100 to 400 mg. per 100 ml.

Again, insulin requirement began increasing. On Oct. 1, 1959, when she received 1,600 units of insulin, prednisone (80 mg. daily) was started. There was a daily drop in insulin need which seemed to stabilize at 200 units daily on the sixth day; at this time prednisone was reduced to 40 mg. daily. During the second and third weeks of prednisone therapy from 150 to 250 units of insulin maintained the blood sugar around 100 to 200 mg. per 100 ml. In the fourth week between 50 to 100 units of insulin were given. Nine weeks after starting prednisone, while taking 50 units of insulin, a three-hour postprandial glucose was 68 mg. per 100 ml. While on prednisone a second episode of pyelonephritis did not produce an increase in insulin need.

On Sept. 15, 1959, serum, on paper electrophoresis, showed increased gamma globulin and decreased albumin. Total serum proteins were 5.6 gm. per cent with 2.0 gm. per cent albumin. There was 8 per cent bromsulphalein retention and a negative cephalin flocculation. Hematocrit was 32 per cent; leukocyte count was 5,000 to 8,400 with 33 per cent polymorphonuclears, 54 per cent band forms, 7 per cent lymphocytes, 3 per cent monocytes, 1 per cent atypical lymphocytes, 1 per cent metamyelocytes and 1 per cent plasma cells. Red blood indices were normal. Reticulocytes were 2.4 per cent. Platelet count was 68,000 to 75,000. No platelet or leukocyte agglutinins were demonstrated by Dr. James Tullis. A bone marrow aspirate showed moderate hypoplasia but no increased iron. Serum iron was 51 μg. per cent with an iron binding capacity of 243 μg. per cent. Hemochromatosis,[2] considered because of insulin resistant diabetes in a postmenopause female, was considered unlikely because of low serum iron in the absence of blood loss and with no increase of iron in the bone marrow. A liver biopsy to evaluate this possibility further was not advisable because of the thrombocytopenia. Stools were negative for occult blood. Lupus erythematosus cells were not demonstrated. Blood urea nitrogen was 14 to 24 mg. per cent, endogenous creatinine clearance 31 ml./min. and an intravenous urogram was normal.

She was discharged on Oct. 26, 1959, weighing 100 pounds on an 1,800 calorie diet. Her daily medications included 100

units of U-500 insulin, 40 mg. of prednisone, 2.0 gm. chloramphenicol, antacids and oral iron. On Dec. 7, 1959, while on 50 units of insulin the blood sugar was 68 mg. per 100 ml. three hours postprandially.

CLINICAL STUDIES

These clinical studies were performed over a six-month period during and following the insulin resistant phase. The results are shown in table 1.

1. *Antibodies to insulin.* Serum from the patient on Sept. 5, 1959, two days after the second episode of diabetic coma, showed faint precipitin bands with commercial beef-pork U-500 insulin (undiluted and diluted 1:10) with the Ouchterlony Technic[2] (courtesy of Dr. John Harter). A similar study on serum of Sept. 18, 1959, while she was taking 1,200 units of U-500 insulin daily, did not show evidence of precipitins.

2. *Insulin binding.* The insulin binding capacity of the serum was determined by means of the resin technic[3] during the months of July, September and December of 1959. The capacity of the serum to bind insulin was considerably elevated during the phase of insulin resistance as evidenced by more than a twentyfold increase in the insulin binding value over that seen during insulin sensitivity. However, during the phase of insulin responsiveness, the insulin binding capacity of the serum was no different from that of the insulin sensitive diabetic patients.

Studies had been done previously in insulin resistant and insulin sensitive diabetic patients to evaluate the possible differences in the uptake and release of I^{131}-labeled insulin by the liver, in vivo. The results clearly indicate that both the hepatic uptake and the release times of the insulin-I^{131} in the resistant patients were considerably prolonged when compared with insulin sensitive diabetic patients.[4] The patient described in this report also exhibited delayed hepatic uptake and slow release of insulin-I^{131}.

3. *Insulin-like activity (ILA) of serum. A. In vitro.* The rat epididymal adipose tissue method described by Martin, et al.[5] was used. In this procedure the amount of $C^{14}O_2$ produced from glucose-1-C_{14} is used as the index of insulin or ILA present in the incubation medium. With this technic undiluted fasting serum from apparently healthy adults[6,7] showed ILA ranging from 0.03 to 0.5 milliunits per ml., with a mean of 0.27. The ILA of insulin treated diabetics varies with the type of insulin, the total amount and the time of insulin administration before the blood sample was taken. For example, in a patient receiving 40 units of NPH insulin daily, serum taken twenty-four hours after the last insulin injection contained 2.0 milliunits per ml. of ILA.

In the present patient, serum was obtained for study of ILA during the period of moderate resistance, two months later at the peak of insulin resistance associated with keto-acidosis, and finally after the return of sensitivity to insulin. Detailed data are presented in table 2. During the resistant phase all sera studied showed markedly elevated levels of ILA. This was especially high during the episode of keto-acidosis (Sept. 3, 1959) and yet considerable additional insulin was needed for adequate treatment. After prednisone therapy the ILA was in the range usual in an insulin sensitive diabetic.

The following evidence suggests that the markedly elevated level of ILA was insulin. 1. At a serum dilution of 1:500 the ILA effect persisted. This makes nonspecific interference unlikely. 2. Anti-insulin serum, induced in guinea pigs with beef insulin, added to the incubation medium caused a marked reduction in ILA. However, the inhibition was not total, perhaps reflecting inhibition of only the beef component of the injected commercial mixture of beef and pork insulin. It is possible that incomplete inhibition at the time of the peak resistance (sample obtained on Sept. 3, 1959) represented saturation of the anti-insulin serum by the excessive amount of insulin present in the patient's serum at that time. Finally, there is the possibility that there was a small amount of endogenous human insulin circulating in the patient's serum.

TABLE 2

Insulin-like activity (ILA) of serum (J.C. No. 54010)

Date	Insulin (U./day)	Assay no.	Dilution	ILA (mu./ml.) Serum	ILA (mu./ml.) Serum + AIS*
7/30/59	450	1	1:32	14.4	1.6
		2	1:32	11.8	
9/3/59	2,500	1	1:32	>16.0	14.7
		2	1:32	>32.0	
		3	1:128	>64.0	
		4	1:500	175.0	
9/24/59	3,200	1	1:128	>64.0†	
12/12/59	50	1	1:32	1.7	
		2	1:8	2.6	1.1

* AIS: Anti-insulin serum prepared in guinea pigs with beef insulin.
† Sample used for in vivo study.

B. *In vivo.* Plasma, 100 ml., which contained in excess of 6.4 units of ILA from the in vitro estimate, was infused into a patient with carcinoma of the lung over seven minutes. The glucose tolerance of the recipient, measured by the rate of disappearance of an infused load of glucose, was in the low normal range ($K = 1.2$).[8] The response of the blood sugar following infusion of the plasma from the insulin resistant donor is shown in figure 3. There is a definite hypoglycemic

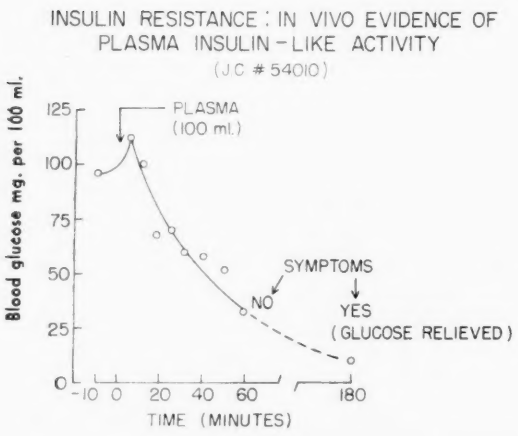

FIGURE 3

response. The peak depression of glucose in the recipient occurred later than one hour after the infusion suggesting a slow but continued release or activation of the insulin-like factor in the patient's plasma.

DISCUSSION

The patient demonstrated a number of clinical features known to be associated with insulin resistance.[28] The resistance per se was a significant factor in the cause of keto-acidosis and coma. Severe keto-acidosis is uncommon in patients with insulin resistance.[25,28] Among thirty-four patients seen at the Joslin Clinic during the last thirty years there are three in whom failure to respond to insulin resulted in coma.[9] Successful treatment required even larger amounts of intravenous insulin and was complicated by an anaphylactic reaction. The tendency of insulin-resistant subjects to develop hypoglycemia for several days after the administration of large amounts of insulin is consistent with the slow dissociation of insulin-protein complexes which have been observed in these subjects.[10,11] This complex of "bound insulin" serves as a depot of insulin which is released in significant quantities over a prolonged period of time.

It is established that antibodies to insulin occur.[29] Yet, these antibodies are not demonstrable by all of the technics for detection of antibodies. For example, immune rabbit serum, which has a high insulin antibody titer by a hemagglutination method, may not show precipitins.[27] The apparent discrepancy with the two studies in the present patient may be explained by antigen excess which can produce a soluble antigen-antibody complex.

Antibodies to insulin and an increased capacity of the serum to bind insulin were demonstrated. Neither of these features has been thought to be specific for insulin resistance.[12-16,30] However, the very high level of binding and the fall following the return of the insulin sensitive state suggest a possible role of the "binding process" in the perpetuation of the resistance. The difference between insulin resistance and nonresistance in insulin-treated patients may be a quantitative one. Berson and Yalow[11] have shown that serum from insulin resistant patients may bind up to 500 units of insulin per liter; they suggest that binding of administered insulin may be the important factor in certain patients with insulin resistance. Lowell,[18,19] Burrows[20] et al. and Vallance-Owen[21] have suggested that the need for large amounts of insulin is to overcome binding or to overcome a circulating insulin inhibitor. Field[22] found that ACTH abolished insulin resistance without altering the ability of the serum to bind large amounts of insulin. The complexity of the binding process, and the difference in technics used for its demonstration, have not permitted definitive evaluation of the role of this factor in insulin resistance.[11]

An increased ILA of serum has been demonstrated in the chronically resistant patient[32,34] and during diabetic acidosis poorly responsive to insulin.[33] In the present instance the high ILA is most likely a measure of the "bound insulin." This in vitro activity may reflect dissociation of insulin from the protein complex. Alternatively, the "binding" may not interfere with active sites of the insulin molecule if the insulin-protein complex can reach the cell. The failure of an in vivo response could be explained by an inability of this insulin-protein aggregate to cross the capillary membrane.

The hypoglycemia following infusion of plasma from the patient into a nondiabetic patient clearly indicates the biologic activity of the insulin. The magnitude of the response is consistent with an in vitro estimate of insulin-like activity made on an earlier serum sample (17.5 units per 100 ml.). The time required for the maximum hypoglycemia is consistent with a delayed release of "bound insulin" in the donor plasma. A slow release is also suggested by the prolonged time required for maximal hepatic uptake of I^{131} from I^{131}-insulin added to the donor plasma.[4] Unfortunately, the functional state of the pituitary, the adrenal and the liver in the recipient could not be evaluated. The low normal rate of glucose utilization observed may represent the impaired glucose tolerance which has been demonstrated in patients with neoplastic disease.[8]

The effect of prednisone, or any presumed specific therapy, is difficult to evaluate because of the variable course of insulin resistance.[28] ACTH[23-25] and prednisone[26] have been reported to decrease insulin requirements in insulin resistant patients. The mechanism for this ap-

parent effect is not clear. In the reported series there was usually a fall in insulin dosage in four to five days and within ten to fourteen days the insulin requirement was less than 100 units daily. This decrease in insulin requirement has been observed in patients with[23] and without hemochromatosis. An effect of adrenal steroids or ACTH on antibody formation has been suggested. In the six patients reported by Oakley et al.[26] the four who responded to prednisone had demonstrable antibodies as measured by the passive cutaneous anaphylaxis test in the guinea pig. The other two did not show antibodies and the insulin requirement increased during prednisone administration. It is difficult to explain the early fall in insulin dose by decreased antibody synthesis since gamma globulins have a half life of the order of fourteen days. This early decrease in insulin could be due to a direct effect on insulin-sensitive cells, an effect on capillary membrane permeability or an alteration of the protein binding. A recent report indicates that methyl prednisolone in a patient with insulin resistance lowered the serum binding globulin concomitantly with insulin.[31]

Figure 2 shows the insulin requirements after starting prednisone in the present patient. The drop to less than 100 units of insulin daily on the third day after prednisone may represent continued release of "bound insulin" following the 3,150 units during the preceding forty-eight-hour period. Over the next three weeks the insulin requirement remained at approximately 200 units per day, lower than at any period since resistance developed five months previously. In the fourth week after prednisone there was a decrease in insulin requirement to less than 100 units daily. By the eighth week, 50 units of insulin daily were adequate to maintain normoglycemia. The final sequence is consistent with an effect of prednisone on antibody formation. Further evidence on the role of prednisone might have been obtained by stopping the steroid. This was not felt advisable because of the severity of the clinical situation during the resistant period. The glucocorticoid property of prednisone did not produce any major problem.[24]

SUMMARY

An instance of insulin resistance of five months' duration is described in which the resistance per se was a major factor in producing diabetic coma. This was successfully treated with large amounts (9,000 units) of insulin. The intravenous insulin produced an anaphylactic reaction and was followed by a five-day period of hypoglycemia. The resistant state returned (1,200 units daily). Prednisone was associated with a return of insulin sensitivity.

Serum from the patient during the resistant period showed precipitin antibodies, increased insulin binding and high levels of insulin-like activity by the in vitro adipose tissue assay. Plasma, 100 ml., infused into a nondiabetic patient produced profound hypoglycemia. The time course of the induced hypoglycemia suggests a slow release of "bound insulin" in the patient's plasma. These observations suggest that the basis for insulin resistance in this instance is an inability of the insulin to reach the cell in a biologically active form at the necessary rate, or a failure of the cell to respond.

SUMMARIO IN INTERLINGUA

Resistentia a Insulina con Alte Nivellos de Activitate Insulinoide in le Circulation

Es describite un caso de resistentia a insulina de cinque menses de duration, in que le resistentia per se esseva un factor major in le production de coma diabetic. Isto esseva tractate a bon successo con grande quantitates (9.000 unitates) de insulina. Le administration intravenose del insulina produceva un reaction anaphylactic e esseva sequite per un periodo de cinque dies de hypoglycemia. Le stato de resistentia recurreva (con un dosage diurne de insulina de 1.200 unitates). Le uso de prednisona esseva associate con le retorno del sensibilitate pro insulina.

Le sero del patiente durante le periodo de resistentia monstrava anticorpore precipitinal, augmento del ligation de insulina, e alte nivellos de activitate insulinoide secundo le essayage in vitro a tissu adipose. Le infusion de 100 ml de plasma ab le patiente produceva profunde hypoglycemia in un subjecto non-diabetic. Le curso del hypoglycemia in le tempore pareva indicar un lente liberation de "insulina ligate" in le plasma del patiente. Iste observationes suggere que le base del resistentia a insulina in le presente caso esseva le incapacitate del insulina de attinger le cellula in un forma biologicamente active e in le quantitate requirite o le incapacitate del cellula de responder.

ACKNOWLEDGMENT

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ADDENDUM

A follow-up* on June 3, 1960, indicates that prednisone was stopped sometime in early 1960. Insulin requirement has progressively risen to the range of 600-800 units daily. This dose level has prevented ketosis;

* Courtesy of Dr. Art. B. Martin, Fort Smith, Arkansas.

fasting blood sugars range between 100 to 300 mg. per 100 ml.

Protein fractions of the patient's serum were separated by column electrophoresis and their effect on glucose uptake of the isolated rat diaphragm tested. Methods used have been reported by Randle, P. J. (*J. Endocrinol. 14*:82, 1956) and Taylor, K. W., and Randle, P. J. (*J. Endocrinol. 19*:221, 1959).

When these protein fractions were dissolved in a volume of buffered glucose-saline thirty times that of the original volume of serum a statistically significant increase in glucose uptake was observed with the gamma globulin fraction *only*. Since these fractions were highly diluted prior to assay the results suggest that a considerable quantity of insulin was associated with the gamma globulins and that this insulin possesses biologic activity in this in vitro system. These observations are reported through the courtesy of Dr. K. W. Taylor.

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The Renal Clearance of I^{131} During the Infusion of NaI^{131} and Insulin-I^{131}

J. William Allgood, Charleston, South Carolina

Although detectable levels of insulin proteases are widely distributed in mammalian tissues, the greatest concentrations have been found in homogenates of liver and kidney.[1] It seems to be well established that the liver degrades insulin and that its "insulinase" activity is not an artifact of sliced and homogenized preparations.[2] Recent work by Stadie, Williams, and others, showed that after the injection of I^{131} insulin into rats, the highest concentration of bound radioactivity was found in the kidney, which suggests that this organ may participate in insulin catabolism.[3,4]

The role of the kidney in insulin degradation is of additional interest in the light of the clinical observation that insulin requirements often decrease in diabetics with advanced intercapillary glomerulosclerosis.[5,6] Although decreased dietary intake may play a role,[7] the existence of decreased insulin catabolism in diabetics with advanced renal disease remains an attractive hypothesis. This study was designed to investigate the role of the human kidney in insulin degradation.

MATERIALS AND METHODS

The purpose of this study was to compare the renal clearance of I^{131} during the infusions of NaI^{131} and insulin-I^{131}. Five patients, two female and three male, whose ages ranged from fifteen to fifty-eight years, and who had no known renal or cardiac disease, were studied. One of the patients had diabetes and had been taking 40 units of NPH insulin daily for ten years. Each of the patients had his thyroid suppressed with two 30 mg. doses of Methimidazole given twelve and three hours before the experiments. Each patient received 1,500 cc. of water twelve and three hours before the experiments, and sufficient water (usually 250 cc. every half hour) to insure a urine flow of at least 2 cc. per minute. The iodide^{131} and insulin-I^{131} infusions were carried out on separate days in random order with at least a six-day interval between the two studies. In determining the renal clearance of I^{131}, a priming dose of 50 μc of NaI^{131}* was injected intravenously, followed by a continuous intravenous infusion of NaI^{131} in normal saline at 0.33 μc/min. The total amount of NaI^{131} administered was approximately 100 μc. This rate was found to be optimal for providing a slowly rising blood level of radioactivity. After a thirty-minute period of equilibration, urine was collected by means of an indwelling Foley catheter for four consecutive thirty-minute periods, and a blood sample was drawn at the midpoint of each period.

In the determination of the I^{131} clearance during the infusion of insulin-I^{131},† a priming dose of 4 μc of insulin-I^{131} was given, followed by a continuous infusion of 0.65 μc/min. of insulin-I^{131} in normal saline. Again, this rate of infusion was found to be optimal for obtaining slowly rising blood levels of radioactivity. Urine and blood were collected in the manner previously described after a thirty-minute equilibration period.

In both types of experiments the infusions were carried out at the rate of 4 ml./minute.

Determinations of radioactivity were made in duplicate on 2 ml. aliquots in a scintillation well counter. Sufficient counts were obtained to maintain a counting error of less than 1 per cent. Samples of urine, whole plasma, plasma TCA precipitates, and plasma TCA supernatants were counted in this manner. The plasma was precipitated with an equal volume of 40 per cent trichloracetic acid. The supernatant was aspirated and counted. The TCA precipitates were washed three times with distilled water and digested with concentrated KOH.

Following NaI^{131} and insulin-I^{131} infusions the radioactivity in the TCA soluble fraction of the plasma was

Winner of the 1959-60 Graduate and Medical Student-Intern Essay Contest of the American Diabetes Association for the best paper in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. This is the paper for which he received his award.

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* Abbott Laboratories.

† Abbott Laboratories — Specific activity = 4.7 millicuries per mg. Concentration: 0.1 mg./ml. Insulin activity: 27 μ/mg. Stored at 4° C. for maximum one week before use.

found to be mainly inorganic I^{131} because it was 97 per cent extractable with chloroform after the addition of iodide carrier and excess iodate.[8] The radioactivity in urine was found to be less than 0.1 per cent precipitable with TCA. In addition, the urinary radioactivity was identical with that of an NaI^{131} standard on descending paper chromatography[2] with a butanol-acetic acid-water solvent (3:1:4) and subsequent autoradiography with Eastman no-screen X-ray film. In one patient after insulin-I^{131} infusion, the urinary chromatograms were divided into 1 to 2 cm.-wide strips and counted in a scintillation well counter. Again, the radioactivity corresponded to the iodide^{131} spot; neither the presence of insulin-I^{131}, labeled monoiodotyrosine, nor diiodotyrosine could be demonstrated.

In both the NaI^{131} and insulin-I^{131} infusions, the renal clearance of inorganic I^{131} was calculated by the following formula:

$$I^{131}\ \text{clearance (ml./min.)} = \frac{\text{Counts/ml. urine} \times \text{urine flow (ml./min.)}}{\text{TCA soluble counts/ml. plasma}}$$

"Endogenous creatinine clearances"[9] were carried out simultaneously in some experiments. No significant differences were observed between values obtained during the NaI^{131} and insulin-I^{131} infusions.

It was assumed that the renal clearance of iodide proceeding from the extrarenal breakdown of insulin-I^{131} should equal the clearance of infused NaI^{131}. If the kidneys effectively bind insulin and participate in its proteolytic destruction, I^{131} may be added to the urine by the kidneys. In this case, the "apparent clearance of iodide" during the infusion of insulin labeled with I^{131} should exceed the clearance of iodide during a NaI^{131} infusion.

RESULTS

Figure 1 illustrates the changes in plasma radioactivity during infusions of NaI^{131} and insulin-I^{131} in the same patient (P.H., twenty-nine-year-old female nondiabetic) on different days.

It can be observed that during the infusion of NaI^{131} practically all the counts are contained in the TCA soluble fraction of the plasma, and the TCA precipitate is negligible. During the insulin-I^{131} infusion, the radioactivity is distributed between the TCA precipitate and the TCA supernatant. The TCA soluble counts rise on a steeper slope than the precipitable counts because the destruction of insulin exceeds the rate of infusion.

Table 1 summarizes the iodide clearances measured during the infusion of NaI^{131} and insulin-I^{131} in five patients.

It can be observed that in all patients the mean clearance of iodide during the insulin-I^{131} infusion was great-

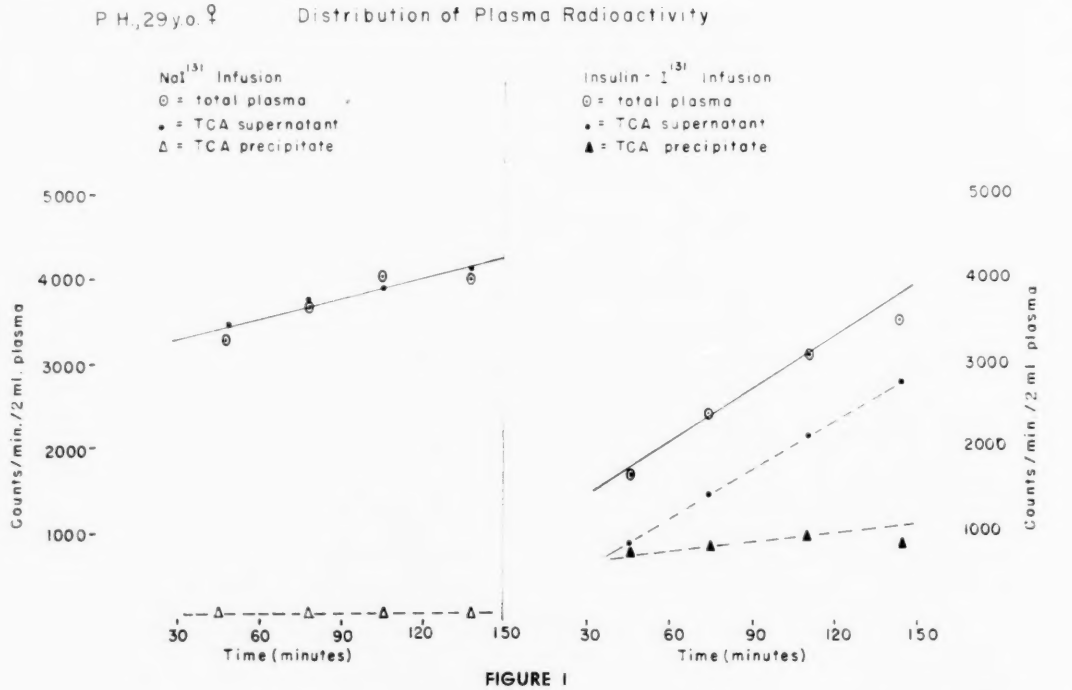

FIGURE 1

TABLE 1

Patient	Collection period	I^{131} clearance (ml./min.) NaI^{131} infusion	Insulin-I^{131} infusion	P
V.D.	1	35.3	50.9	
Age 50	2	34.8	47.6	
Male	3	30.7	40.7	
	4	22.1	44.1	
	Mean±S.E.*	30.7±3.8	45.8±2.2	<0.01
J.M.	1	—	60.2	
Age 36	2	40.1	65.7	
Male	3	44.1	64.6	
	4	44.7	58.1	
	Mean±S.E.	43.0±1.4	62.1±1.8	<0.05
F.W.	1	43.8	43.4	
Age 15	2	34.1	48.5	
Female	3	38.4	40.6	
	4	42.7	51.6	
	Mean±S.E.	39.8±2.2	46.0±2.5	<0.2
A.W.	1	36.8	42.8	
Age 58	2	32.9	44.3	
Male (diabetic)	3	28.2	40.2	
	4	27.0	37.9	
	Mean±S.E.	31.2±2.2	41.3±1.4	<0.01
P.H.	1	37.6	30.4	
Age 29	2	—	43.8	
Female	3	34.8	51.4	
	4	27.5	39.6	
	Mean±S.E.	33.3±2.4	41.3±4.4	<0.2
Average Mean±S.E.		35.6±2.5	47.3±3.8	<0.05

* S.E. = standard error of the mean.

er than the mean clearance of iodide during the infusion of NaI^{131}. In three patients, (J.M., V.D., A.W.) this difference was statistically significant. In two patients, (F.W. and P.H.) there was some overlay, but the trend was the same. The mean clearance of iodide in the five patients was 35.6 ml. of plasma/min., while the mean of the apparent clearance of iodide during the infusion of insulin-I^{131} was 47.3 ml. of plasma/min. The statistical probability of the differences between these means being due to chance is less than 5 per cent. When paired analysis of the five pairs of clearances is carried out, the probability of the differences being due to chance is less than 1 per cent.

Figure 2 compares the levels of TCA precipitable and soluble radioactivity during the infusion of insulin-I^{131} in a representative nondiabetic patient (F.W.) and a diabetic (A.W.). The latter patient had been taking 40 units of NPH insulin daily for the last ten years. In the nondiabetic, the rate of destruction of insulin-I^{131} exceeded the rate of infusion shown by the steeper slope of TCA soluble radioactivity as compared to the protein bound counts. When insulin-I^{131} was infused at the same rate to the diabetic patient the following differences were observed: (a) the levels of protein-bound radioactivity in plasma were higher than in any of the nondiabetic patients studied; (b) the rate of accumulation of protein bound radioactivity was greater, resulting in a steeper slope of the TCA precipitable counts; (c) the rate of destruction of insulin-I^{131} was decreased, as illustrated by the slower rise of TCA soluble radioactivity. These findings are best explained by the presence of insulin binding antibodies in the plasma of the diabetic patient treated with insulin.[10,11]

Although patient A.W. probably had circulating insulin binding antibodies, it is of interest to note that similarly to the nondiabetics, his "apparent clearance" of I^{131} during the infusion of insulin-I^{131} exceeded that observed during the infusion of NaI^{131}.

In three additional patients, an attempt was made to obtain a decrease in the apparent iodide clearance by diluting the infused insulin-I^{131} with nonlabeled insulin. Figure 3 illustrates the results of one of these experiments which were essentially the same as those obtained with the other two patients.

In these experiments, 100 microcuries of insulin-I^{131} were infused over a three-hour period at a rate of 0.0002 units of insulin per minute. After ninety minutes, the labeled insulin was diluted with a simultaneous infusion of 0.1 or 0.3 units of nonlabeled insulin* per minute (the nonlabeled insulin exceeding the labeled insulin by a ratio of 500-1,500:1). The rate of infusion was 4 ml./min. during the entire experiment. The solvent was 0.9 per cent saline during the first, and 5 per cent glucose during the second, experimental period.

The upper part of figure 3 shows that there was no break in the slope of inorganic iodide (TCA supernatant) which should be expected if the sites of insulin binding had been saturated and insulin breakdown inhibited by the infusion of nonlabeled insulin. The lower part of figure 3 demonstrates that there was no decrease in the apparent iodide clearance.

DISCUSSION

Our working hypothesis is that the kidney clears iodide derived from the extrarenal breakdown of insulin-I^{131} in the same manner as it clears infused sodium iodide. If this is correct, the apparent increase in iodide clearance during the infusion of insulin-I^{131} is a result of addition of iodide to the urine by the kidneys. This iodide could proceed either from the labeled insulin itself or from one of its circulating breakdown products from extrarenal sites. Iodinated insulin gives rise to monoiodotyrosine (MIT) and diiodotyrosine (DIT),[2] both

*Crystalline Zinc Insulin, Lilly.

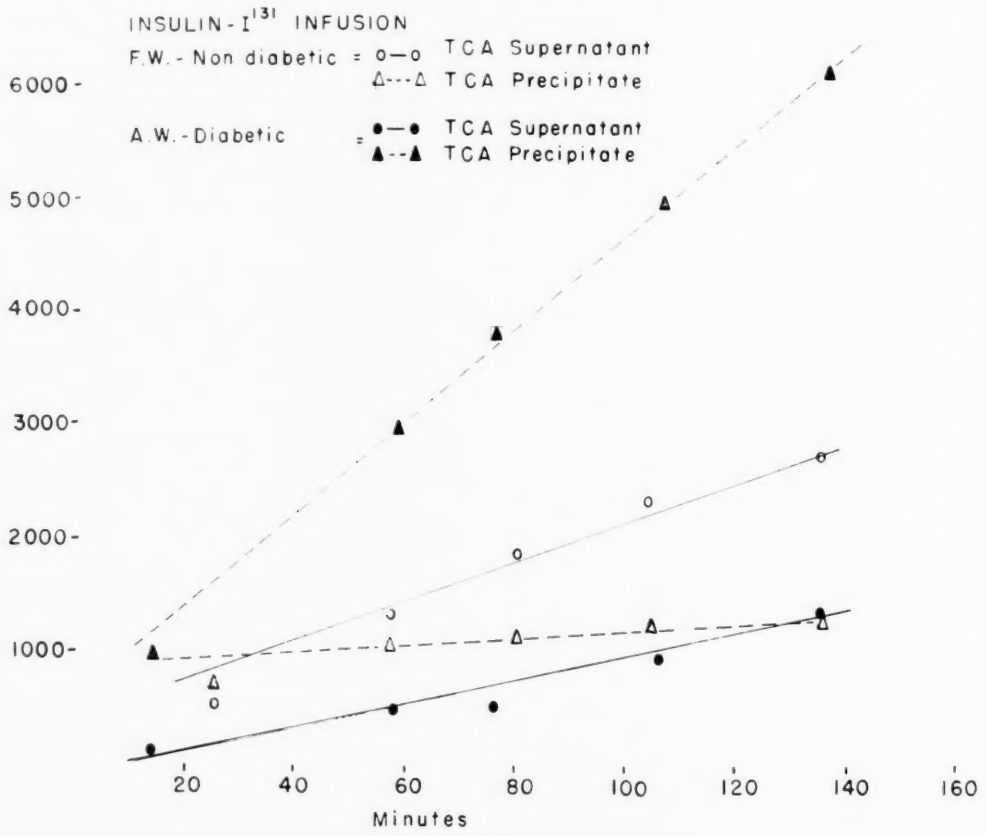

FIGURE 2

of which are deiodinated by renal tissue in vitro.[12] Lassiter and Stanbury[13] studied quantitatively the renal clearance and deiodination of infused DIT in humans. Their figures indicate that the amounts of DIT^{131} circulating in plasma at any given moment should be approximately half as much as that present in the form of $iodide^{131}$ to explain the observed increase in iodide clearance during the infusion of insulin-I^{131}. However, the circulating amounts of DIT and MIT during insulin-I^{131} infusions, if present at all, are extremely small mainly due to the rapid hepatic deiodination of the tyrosines.[2]

An attempt was made to block competitively the binding sites of insulin-I^{131} in the kidney by diluting the insulin-I^{131} infusion with nonlabeled insulin (figure 3). The failure to accomplish this was probably a result of not administering enough insulin. Prout and Evans[14] showed that in rats more than 100 units of insulin/kg. are necessary to achieve saturation of binding sites and decreased insulin catabolism.

The possibility of labeled and nonlabeled insulin having different degradation pathways has been shown to be unlikely by work with intact mice,[15] eviscerated nephrectomized rabbits,[16] liver extracts,[17] and perfused rat

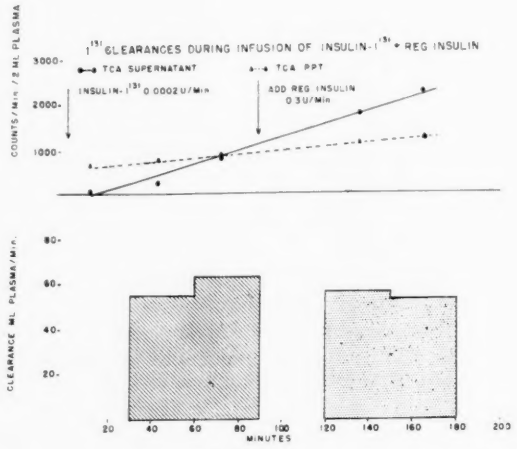

FIGURE 3

livers.[2] Further study of the I^{131} renal clearances of diabetics with intercapillary glomerulosclerosis will be necessary before the clinical implications of the renal breakdown of insulin can be evaluated.

SUMMARY

The renal clearance of iodide^{131} was studied during continuous infusions of NaI^{131} and insulin-I^{131}. It was assumed that the renal clearance of iodide proceeding from extrarenal breakdown of insulin-I^{131} would equal the clearance of infused NaI^{131}. In all experiments, the "apparent iodide clearance" during insulin-I^{131} infusion was greater than the clearance of infused NaI^{131}. The mean iodide clearance of five patients was 35.6 ml./min. $\pm$ 2.5, while the "apparent iodide clearance" during insulin-I^{131} infusion was 46.3 ml./min. $\pm$ 3.8 ($p<0.05$). It is probable that the excess I^{131} that appeared in the urine during the infusion of insulin-I^{131} proceeded from degradation and deiodination of hormone concentrated in the kidney.

Dilution of insulin-I^{131} by simultaneous infusion of 0.4 U./kg. unlabeled insulin did not change the "apparent I^{131} clearance."

SUMMARIO IN INTERLINGUA

Le Clearance Renal de I^{131} Durante le Infusion de NaI^{131} e de Insulina-I^{131}

Le clearance renal de ioduro^{131} esseva studiate durante le continue infusion de NaI^{131} e insulina-I^{131}. Esseva supponite que le clearance renal de ioduro resultante ab le decomposition extrarenal de insulina-I^{131} deberea esser equal al clearance del infundite NaI^{131}. In omne le experimentos, le "apparente clearance de ioduro" durante le infusion de insulina-I^{131} esseva plus grande que le clearance del infundite NaI^{131}. Le clearance medie de ioduro de cinque patientes esseva 35,6 $\pm$ 2,5 ml/min, durante que le "apparente clearance de ioduro" durante le infusion de insulina-I^{131} esseva 46,3 $\pm$ 3,8 ml/min ($p<0,05$). Il es probabile que le excesso de I^{131} que appareva in le urina durante le infusion de insulina-I^{131} resultava de degradation e disiodisation de hormon concentrate in le renes.

Le dilution de insulina-I^{131} per le infusion simultanee de 0,4 unitates per kg de peso corporee de non-marcate insulina non alterava le "apparente clearance de I^{131}."

ACKNOWLEDGMENT

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Spontaneous Diabetes Mellitus in the Chinese Hamster (*Cricetulus Griseus*)

II. Findings in the Offspring of Diabetic Parents

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Recently the occurrence of spontaneous diabetes mellitus in the Chinese hamster (*Cricetulus griseus*) was reported by us.[1] Apart from the fact that spontaneous diabetes is unusual in rodents, the similarity of the pathological findings to certain changes in humans and in experimental animals seemed of particular interest. The condition is primarily pancreatogenic, the main cause of diabetes in the Chinese hamster being a disease of the beta cells in the pancreatic islets, consisting of karyolysis, glycogen infiltration and disappearance. Concurrently, renal glomerular changes resembling those of alloxan-treated animals were observed. These consist of severe ischemia, mostly diffuse intercapillary precipitations of hyaline, PAS positive material, obliteration of the usual glomerular structure, deprivation or swelling of endothelium, thickening of the Bowman's capsule and often adhesion of it to the capillary tufts. Evidence was cited that in the Chinese hamster, the disease is genetically determined. Apparently the trait is transmitted as a recessive factor, with varying degrees of penetrance in certain families of four major inbred lines.[1,2] The mode of inheritance is being investigated and will be the subject of a separate report. It is obvious that there is tremendous value in having at hand a spontaneously diabetic animal for genetic and metabolic studies, as well as for testing of potentially hypoglycemic agents.

The present paper describes the pathologic findings in the progeny of diabetic parents and some aspects of our breeding program. Data on insulin assays, indicating varying degrees of deficiency, electrophoretic studies and preliminary observations on chemotherapy, will be reported later.

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BREEDING

Although recognized in our colony only in 1957, there are indications that the disease occurred as early as 1954. It arose spontaneously during the course of inbreeding. At that time, many of the sublines of the major families were approaching the fourth generation of continuous brother-sister mating. The incidence of polyuria and polydipsia, two of the most prominent symptoms, rose gradually during the ensuing years. Following establishment of a pathologic diagnosis, an extensive breeding program was inaugurated to evaluate the genetic background. Four inbred lines (JFY, VSY, ORY and JBY) were affected, with high incidence (65-90 per cent) and great severity. As the number of diabetic hamsters has increased to between 100 and 200 at the present time, sufficient evidence is now at hand to indicate the role of one or more recessive genes in the homozygous state. The possibility that an unknown number of genes may be taking part in the expression of the disease still exists, since an association of specific genes is expected in the Chinese hamster because of the small number of chromosomes ($2n = 22$). Although the participation of a recessive gene is indicated, the degree of penetrance (or intensity of suppression) is enhanced when the over-all genetic background of the animals reaches 65 per cent, or higher, homozygosity. Random bred animals having 50 per cent homozygosity fail to show diabetes; inbreeding from the fourth to the eighth brother-sister generation (JBY family) has raised the incidence of diabetes to 90 per cent of the offspring. Since the degree of penetrance or severity of the disease varies among families, the over-all genetic constitution of a litter governs the degree to which the metabolic disturbance is expressed.

CLINICAL AND LABORATORY FINDINGS

Test sticks* for detection of urine sugar and ketone bodies proved extremely helpful in early diagnosis of incipient diabetes. They are also convenient in evaluating hypoglycemic therapy, and in establishing effective drug dosages. Each of the diabetic lines varies in the age of onset and incidence of hyperglycemia (depending upon state of inbreeding). Diabetes may appear as early as eighteen days or as late as 250 days of age. The intake of food and water increases two- to threefold, and many animals appear bloated from distention of the digestive tract. The incidence among litter mates ranges from 65 per cent to 90 per cent; litter mates generally display similar levels of glucose and ketone bodies and respond equally to therapy. This feature permits replication of trials or multiple therapy on both sexes.

PATHOLOGY

Offspring of diabetic parents and of normal control matings were sacrificed at daily intervals from two hours of age to fourteen days, and also at 18, 23 and 35 days. No gross abnormalities were noted except in the progeny of the VSY lines. The macroscopic findings, as observed in adults of this family, were restricted to the urinary system. The pelves and calyces of kidneys were bilaterally distended (hydronephrosis), detectable as early as two to three days after birth and becoming gradually more severe with age (severely polyuric).

Body weights and lengths were obtained from approximately twenty litters of both diabetic and normal parents; no significant differences were noted. The gestation period was the same in both groups.

The average litter size of all animals cared for and growing up, one week after birth, was between four and six, and was comparable to that of normal parents; a total of seventy-one litters, representing three generations of diabetic matings (fourth to seventh generation inbreds) and seventy litters from healthy parents were compared. Among diabetics, abortions, incomplete pregnancies and deaths at delivery were encountered. Apparently newborns were lost due to cannibalism, especially in the larger litters; sometimes entire litters were eaten. If the estimated loss due to various causes, of about 20 per cent of diabetics' offspring is added, diabetic parents produce larger numbers of offspring than inbred or hybrid normal parents.

Microscopic sections were routinely stained with hematoxylin-eosin; special stains employed were chrome-alum hematoxylin phloxin, aldehyde fuchsin, PAS, and Sudan-hematoxylin. Tissues were fixed in Bouin's solution and cut at 8μ, except for frozen sections, which were cut at 12 to 16μ. Due to the extreme difficulty of sectioning the pancreas in such small animals, especially when only a few hours to three or four days of age, entire animals were blocked. In order to judge numbers and sizes of the pancreatic islets, serial sections were required. Islet measurements and differential counts of alpha and beta cells were obtained to assess possible differences between diabetic and normal parents' progeny.

Pancreas. Sequential comparisons, with increasing age, of islet numbers, sizes and alpha:beta cell ratios disclosed striking differences between offspring of diabetic, prediabetic and normal parents. The great majority of diabetics' offspring revealed islets which, characteristically, were more numerous, larger (due to hyperplasia) or both (figure 1). Islets regarded as hyperplastic consisted almost entirely of beta cells. Formation of islets appeared to occur from small ducts, the islets forming cuffs around them or incorporating them. At two hours of age, proliferation as indicated by the presence of mitotic figures seemed more pronounced in the diabetics' offspring than those of prediabetic* or normal parental mating (figure 2). At one day of age, the islets in progeny from diabetic parents were about twice as large (approximately 100μ) as in normal controls (50μ) and were about four times as numerous.

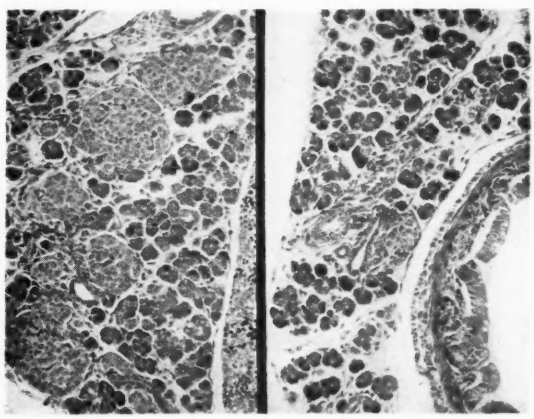

FIG. 1. Islets are more numerous and larger in progeny of diabetic parents (left) than in normals (right), one day old. H.E. X 79.

*Ames Company, Inc., Elkhart, Indiana.

*"Prediabetic" mating refers to crosses between parents prior to their becoming clinically diabetic. Since the age of onset of diabetes is fixed in each line and predictable, brother-sister matings could be made while diabetes was still unapparent.

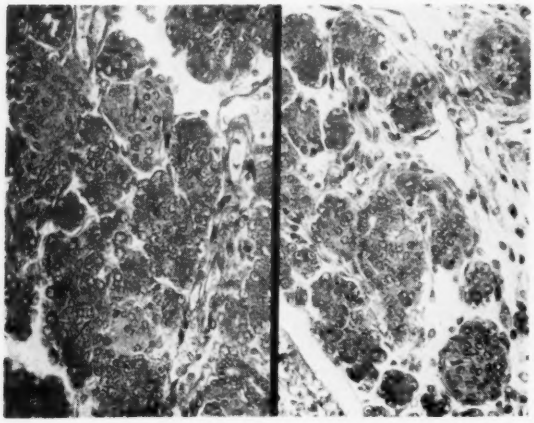

FIG. 2. At two hours of age, proliferation as assessed by mitotic figures is more pronounced in offspring from diabetic parents (left) than in those of normal parent matings. H.E. X 160.

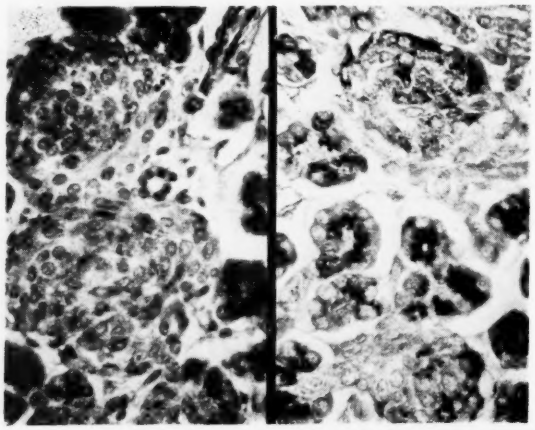

FIG. 3. Sections depicting largest diameter of islets from young of diabetic (left) and of normal parents (right); the beta cells are finely granulated. Aldehyde-fuchsin X 304.

Cell counts per islet (counting all cells in the plane of the largest diameter) averaged 120 cells in offspring of diabetic animals and forty in those of normals; alpha: beta cell ratios, as obtained in sections stained with aldehyde fuchsin, were found to be 1:7 (diabetic) and 1:5 (normal), respectively. The beta cells were finely granulated and the granulation was evenly distributed throughout their cytoplasm (figure 3). Islets of offspring from matings of prediabetic animals showed no difference when compared with normals. At five to seven days, comparisons of the islets in the progeny of diabetic and normal animals disclosed smaller differences relative to number, size and alpha:beta ratios and at ten to fourteen days they were almost completely abolished (figure 4). The diameter of the islands measured roughly 75 to 120μ and alpha:beta cell ratios were 1:4, as in normal adult animals. While the islets were still proliferating as evidenced by mitotic activity, their growth occurred between five and fourteen days (more rapidly in the normal progeny), mitotic indexes being about three to five per islet against one or two in the prediabetic progeny (figure 5). The beta cell granulation had become abundant, essentially filling the entire cell.

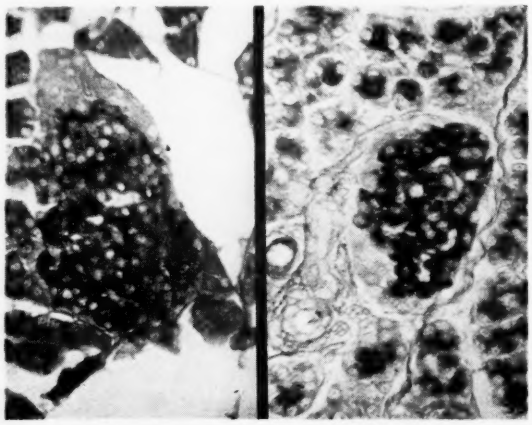

FIG. 4. At seven days, in both normal (right) and prediabetic animals (left) the granulation is more dense; differences still exist in the alpha-beta ratios, absolute numbers and islet sizes. Aldehyde-fuchsin X 304.

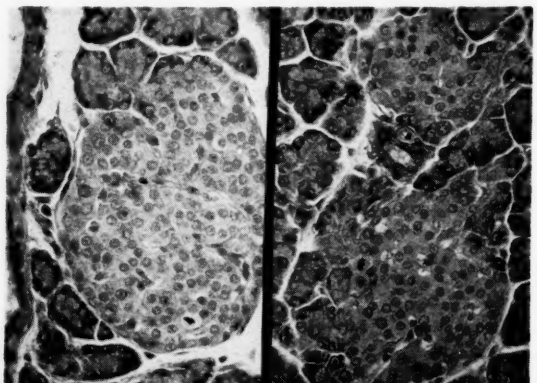

FIG. 5. Mitotic activity persists in islets of both prediabetic (left) and normal animals (right), but is greater in the former (ten days). H.E. X 304.

No increase in the size of the islets was noted at

twenty-three days or older, except in one family line of diabetic parents (VSY). This strain was completely inbred, all progeny revealing clinical diabetes at eighteen days of age. At fourteen days, secondary islet cell hyperplasia occurred, associated with beta cell degranulation; a few cells showed a hydropic change, also (figure 6). In other families, degranulation took place gradually, requiring weeks; the islets were either almost totally degranulated or had only a few well granulated cells left. Large intracellular vacuoles were noted, with strands crossing them and staining, in part, PAS-positive (figure 7). This material has been identified as glycogen both by additional histochemical procedures,* and electron microscopy.†

Liver. Active hematopoiesis persisted for one to two weeks after birth. Very little fat and glycogen were present either in normal controls or in the prediabetic young.

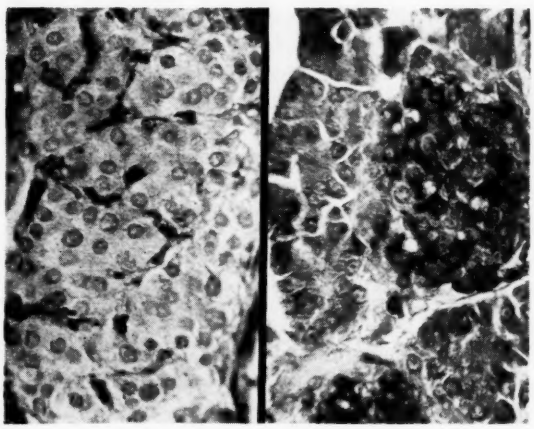

FIG. 6. In the VSY line at fourteen days there is a secondary hyperplasia of islets associated with beta cell degranulation (left); a few cells may also show hydropic change. Clinical diabetes manifests itself within four days (day eighteen). A normal control, fourteen days, is shown for comparison (right). Aldehyde-fuchsin X 304.

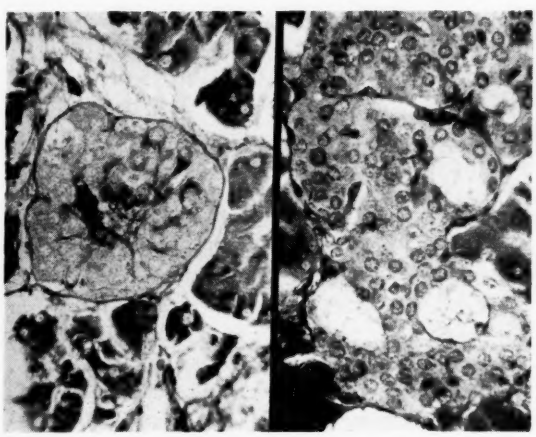

FIG. 7. In other families, degranulation takes place gradually, affecting fewer cells and requiring weeks. An islet of an animal with early clinical diabetes is depicted (left), still having a few well granulated cells left and one with severe diabetes of about one month, showing large intracellular vacuoles and strands containing PAS-positive material (right). Aldehyde-fuchsin X 304.

* By Dr. Samuel S. Spicer of the National Institutes of Health, and

† By Dr. Joseph Williamson of Washington University, to both of whom we are deeply indebted.

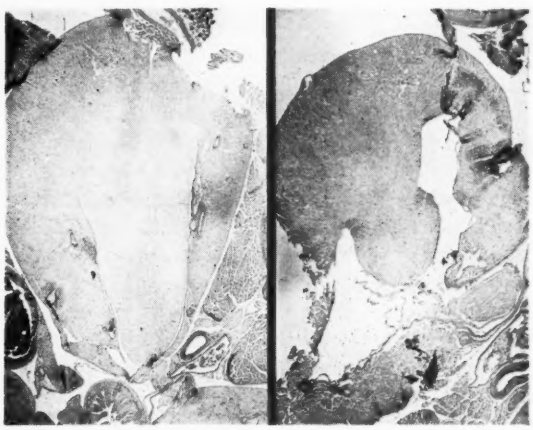

FIG. 8. Congenital hydronephrosis is present in all members of the inbred VSY line. A sagittal section of a kidney showing moderate dilation of the pelves and calyces from a VSY line animal (right) in comparison with one from a normal (left). Aldehyde-fuchsin X 2.7.

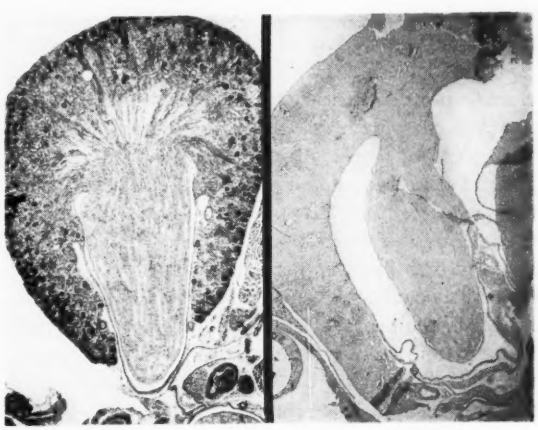

FIG. 9. The dilatation of the calyces progresses with age (right); the difference from a normal is depicted (left). Age seven days. H.E. and aldehyde-fuchsin X 2.7.

Kidneys. In all members of the VSY line there was, progressing with age, moderate to severe dilation of the pelves and calyces in the absence of parenchymal compression and urinary tract obstruction (figures 8, 9). No other pathologic findings, e.g., PAS-positive deposits in glomeruli, were detected. Small scattered foci of erythropoiesis occurred in the kidneys of both groups of animals; the glomeruli appeared mature at about one week of age.

Other Tissues. All other organs sectioned were normal. In view of the small size of the animals some of the endocrine glands, e.g., pituitary, could not be examined.

DISCUSSION

The two outstanding histologic findings in progeny from diabetic parents were hyperplasia and increased size and number of the pancreatic islets. In some instances, especially the descendants of the VSY line, almost entire low-power fields consisted of islet tissue. The majority of cells were identified by special staining technics (aldehyde fuchsin; chrome alum hematoxylin phloxin) as beta cells. The association of ductular structures with hyperplastic islets suggests formation of these from small ducts. Hyperplasia of islets in infants of diabetic mothers has also been recognized in man. However, infiltration by eosinophilic leucocytes (interpreted as extramedullary hemopoiesis or exudate), observed in newborn babies, has never been observed in the hamster.[2] In man, among the less probable causes, hyperplasia has usually been considered as being due to maternal hyperglycemia, the degree reflecting the severity of the mother's diabetes (for review, see reference 3). Other evidence cited favoring this hypothesis stemmed from experimental work, e.g., the induction of islet cell hyperplasia in rats in response to hyperglycemia.[4] It seems noteworthy, however, that in the Chinese hamster some degree of islet hyperplasia occurred in offspring of parents with normal blood sugars (hypoglycemic therapy); many animals were totally dependent upon therapy for continued existence and maintenance of fertility. The mechanism underlying islet hyperplasia, therefore, is still obscure. Further studies are needed to determine the relationship of degree of hyperplasia and severity of parental diabetes, the influence of the prediabetic state, and therapy. Soon after birth, in the normal offspring rapid islet proliferation ensues, abolishing differences existing between them and the prediabetic progeny in the first few days to one week of life. The period of apparent normalcy prior to the onset of degenerative changes (i.e., hydropic degeneration, deposition of PAS-positive material, leading to an eventual beta cell deficiency) varied greatly with each line.

Macrosomia and visceromegaly (particularly cardiac), well-recognized findings in human babies of diabetic mothers, have not been observed in the Chinese hamster.[5] It should be noted, however, that although the number of offspring is comparable to that of normal litters, the figures quoted represent reduced litter sizes. Abortions, death at delivery, and cannibalism were encountered, and the loss of animals was estimated at about 20 per cent. The number of fetuses is significantly greater than in nondiabetics. The effects of hypoglycemic therapy on fertility could not be assessed adequately.

The problem of fetal mortality and macrosomia in experimental animals has been investigated by others. Diabetes induced with alloxan in pregnant rats resulted in a high incidence of fetal death, absorption and abortions, but no large sized offspring.[6,7] In contradistinction, rats partially pancreatectomized during pregnancy and treatment of pregnant animals with growth hormone, produced giant fetuses; hyperplasia of Langerhans islets was absent.[8,9]

In view of the forthcoming paper on genetic aspects, they will not be dealt with further at this time (see above). Hereditary aspects of diabetes mellitus in man are not yet clear.[10,11] The size and composition of the human material greatly influence the results, which are difficult to interpret and are misleading. Obviously, there is tremendous value in having at hand an experimental animal in which genetic studies can readily be made.

Of interest is the observation of hydronephrosis in the VSY line, detectable as early as two to three days after birth. In the absence of any evidence of mechanical urinary obstruction (congenital stricture of the urethra as reported in humans) and compression of the renal parenchyma, it is to be considered as another genetically determined expression. Since in man it has generally been stated that congenital anomalies are more common in infants of diabetic mothers, the finding lends support to this possibility.[5]

Attention has also been focused on tissues other than pancreas, e.g., endocrines (especially the adrenals in view of their relation to carbohydrate metabolism), lymphoid tissue, bones and cardiovascular system. No significant changes were found, with the possible exception of the liver. In the absence of fat and glycogen, vacuolization, particularly in perinuclear zones, is unexplained. Similar findings were described in adult diabetic hamsters, although it appeared that they had increased glycogen deposits compared with adult con-

trols. This finding was somewhat bizarre and should be checked by quantitative analysis. Increased amounts of glycogen in the heart and "glycogen nephrosis" (glycogen deposits in the loops of Henle), demonstrated by the not too reliable Best carmine technic in some human fetuses of diabetic mothers, did not occur in the hamster.[12]

Usually diabetes mellitus manifests itself only in adult life (over four to six weeks of age) in the hamsters (JFY, ORY, JBY), except for a mild to moderately severe form in one line (VSY) appearing as early as eighteen days. The latter might be considered as a juvenile type (however, hereditary) morphologically comparable to certain cases of diabetes in children. A rapid sequence of islet changes occurs over a four-day period, between fourteen and eighteen days. So-called congenital diabetes (e.g., from lack of islands, maturation arrest with failure of alpha cells to convert into beta cells) as described in man has not been observed in the Chinese hamster.[12] It remains to be ascertained in the Chinese hamster at what stage of fetal development the islet hyperplasia begins.[13] In view of increased mortality (infection, deformities), among liveborn human infants from diabetic mothers it also seems desirable to follow up the natural death rate among offspring of diabetic parents.[14]

SUMMARY

A brief history of the origin of diabetes mellitus in certain lines of Chinese hamsters has been presented. Evidence was cited which indicates that the condition is hereditary and possibly transmitted by a recessive trait. In prediabetic hamsters, the islets are abnormally large and numerous. The hyperplasia affects only beta cells, and many islets are in transitional continuity with small ducts. During the first weeks of life, islet cell proliferation (mitotic index, islet size increase) occurs at a more rapid rate in normal controls than in prediabetic animals. Thereafter, for a variable length of time (depending upon the line of hamsters) the findings in the islets in both groups are comparable, but in the prediabetics degeneration of beta cells ensues and leads to eventual deficiency in a large portion of offspring (state of inbreeding) from four major sublines (JBY, ORY, JFY, VSY). Hydronephrosis was found to be an additional genetically-controlled expression in one line (VSY). Macrosomia and visceromegaly (either macro- or microscopic) were not observed. However, the number of fetuses carried by diabetic mothers was larger than in normal animals. The litter sizes (at one week) composed of prediabetic hamsters were about the same as the controls (? physiological limit) due to abortions, deaths at delivery, and cannibalism.

SUMMARIO IN INTERLINGUA

Spontanee Diabete Mellite in le Hamster Chinese (Cricetulus griseus). II. Observationes in le Prole de Parentes Diabetic

Es presentate un breve historia del origine de diabete mellite in certe lineas de hamsters chinese. Es citate constatationes que indica que le condition es hereditari e possibilemente transmittite per heterozygositate recessive. In hamsters prediabetic le insulas es anormalmente grande e numerose. Le hyperplasia affice solmente cellulas beta, e multes del insulas se trova in continuitate transitional con micre ductos. Durante le prime septimanas del vita, proliferation del cellulas de insula (indice mitotic, augmento del dimensiones del insulas) occurre plus rapidemente in normal animales de controlo que in le animales prediabetic. Postea—durante un variabile intervallo de tempore (in dependentia del linea de hamsters in question)—le constatationes in le insulas in le duo gruppos es comparabile, sed in le prediabeticos un degeneration del cellulas beta seque e resulta in le curso del tempore in deficientias in un alte proportion del prole ab quatro major sub-lineas (JBY, ORY, PFY, VSY). Esseva trovate que hydronephrosis occurreva additionalmente como expression genetic in un linea (VSY). Macrosomia e visceromegalia non esseva observate macro- o microscopicamente. Tamen, le numero del fetos portate per matres diabetic esseva plus grande que in femininas normal. Le numeros in le portatas al fin del prime septimana in le caso de hamsters prediabetic esseva approximativemente le mesmes como in le casos de controlo (limite physiologic?) como resultato de abortos, mortes in parturition, e cannibalismo.

ACKNOWLEDGMENT

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The authors wish to acknowledge with gratitude the interest and many favors rendered by Dr. Philip M. LeCompte, of the Faulkner Hospital and Harvard Medical School.

ADDENDUM

In view of a recent paper entitled "Progressive Intercapillary Glomerulosclerosis in the Mouse, Rat, and Chinese Hamster, Associated with Aging and X-ray Exposure," by P. H. Guttman and H. I. Kohn (*American*

Journal of Pathology 37:293-308, 1960) describing a form of diffuse intercapillary glomerulosclerosis as an expression of age rather than of a specific disease, certain comments are necessary regarding the glomerular changes referred to in this paper. Although there is no argument as to the occurrence with age of some morphologic alterations in renal glomeruli of Chinese hamsters as described by Guttman and Kohn and also observed by us, they differ strikingly from those observed associated with spontaneous hereditary diabetes mellitus, both qualitatively and quantitatively. In diabetic intercapillary sclerosis we find significant thickening or splitting of the basement membranes and intercapillary deposition of PAS-positive hyaline masses. The extent of the lesions correlates both with time of onset and severity of diabetes, e.g., in one line of hamsters with diabetes characteristically manifest at day eighteen of life, intercapillary glomerulosclerosis develops parallel and in progression with the diabetic condition as judged by histologic examination of the pancreatic islands and glucosuria. The most severe degree of glomerular change may be seen at one month of age, many animals dying as a result of uremia. Age most certainly is of inconsequential influence. A detailed account of the renal lesions as related to diabetes is contemplated (Meier).

It is perhaps superfluous to point out to the uncritical reader that Guttman and Kohn have examined only four animals (BUY-line, two aged sixty-six days and two 745 days old); also if they had indeed found some similarity to diabetic intercapillary glomerulosclerosis it should be stated that the BUY-line has a genetic background for diabetes. The point to be made here is that perhaps the renal lesion is primary and not necessarily consequential to beta-cell degeneration. In this connection another observation of ours should be mentioned relating to the finding of increased alpha-2 levels, two to three times the normal values, in serum electrophoretic patterns of families with a high incidence of spontaneous diabetes. Upon random hybridization of high incidence families by single or double crosses normal values of alpha-2 are re-established. Since alpha-2 serum proteins are increased even *prior* to the onset of clinical diabetes, breeding experiments have been initiated using elevated alpha-2 serum proteins as a genetic marker in attempts to detect a prediabetic state. Elevated alpha-2 levels may not be due to an increase of normally existing alpha-2 proteins but perhaps to new and different molecules peculiar to the diabetic condition. The results of these studies will be published in the near future (M. N. Green, G. Yerganian and H. Meier).

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Spontaneous Hereditary Diabetes Mellitus in the Chinese Hamster (*Cricetulus Griseus*)

III. Maintenance of a Diabetic Hamster Colony With the Aid of Hypoglycemic Therapy

Hans Meier, D.V.M., Ph.D., and George Yerganian, Ph.D., Boston

Spontaneous diabetes mellitus, as observed in Chinese hamsters, has recently been described.[1] Apart from the fact that spontaneous diabetes is unusual in a rodent species, the similarity of the pathological findings to certain human changes and (alloxan-induced) diabetes in experimental animals is of particular interest. The disease is primarily pancreatogenic, the pathological changes consisting of severe damage, vacuolar ("hydropic") degeneration, and deficiency of beta cells; this is borne out by insulin assays.[2] A concurrent pathological finding is diffuse intercapillary glomerulosclerosis associated with deposition of PAS-positive staining material, and leading to uremia in some animals.[1] Polyuria and polydipsia are usually the most prominent clinical signs; some hamsters excrete up to 70 ml. of urine in twenty-four hours.

Considerable evidence has accumulated to indicate that the disease is hereditary. Diabetes arose spontaneously during the course of inbreeding at a time when many of the sublines of four major families approached the fourth generation of continuous brother-sister mating. The average frequency of genic homozygosity was estimated to be 65 per cent at that time. Attempts at maintaining an extensive breeding program for diabetic hamsters have, so far, been successful. To date, four generations of diabetic hamsters have been obtained with the incidence of diabetes increasing sharply from the fourth to eighth generation, up to 90 per cent of the offspring (e.g., JBY family) becoming diabetic later in life. The degree of penetrance, severity of the disease and age of onset vary greatly among families.[3] Maintenance of fertility of diabetic parents was an ever present obstacle. Also, there were losses of up to 20 per cent of the animals during pregnancy (abortions, resorption) and live-born animals (death soon after delivery, cannibalism). Repeated daily mating tests and treatment considerably improved the number of pregnancies and successful deliveries since the estrus cycles of each female varied greatly. Many animals are totally dependent upon careful control of diabetes for continued existence and maintenance of fertility.

The present report deals with preliminary pharmacologic studies of three hypoglycemic agents, NPH insulin, phenformin, and tolbutamide in both normal and diabetic hamsters. Special emphasis will be placed on experience gained in maintaining a colony of diabetic hamsters for the study of their genetic background. (In addition, other potentially hypoglycemic agents, e.g., thioglycolic acid[4] are being studied.)

EXPERIMENTAL

A. *Laboratory Tests.* Clinical biochemical tests were originally restricted to basic diagnostic determinations, such as blood and urine glucose, NPN, urine ketone bodies and specific gravity. Blood glucose levels for normal hamsters average 110 ± 6 mg. per 100 ml.; for diabetic hamsters between 200 and 800 mg. per 100 ml. The specific gravity of urine is greatly elevated in the glycosuric animals, from 1,018 to abnormal, 1,029++. In hamsters showing signs of uremia, blood NPN levels over 100 mg. per cent are found, normal being 45 ± 3 mg. per cent. In attempts to determine whether or not an animal is diabetic, rapid qualitative or semiquantitative tests (test sticks*) are conducted routinely. These prove especially helpful in early detection of diabetes and also in evaluating hypoglycemic therapy and establishing effective drug dosages.

B. *Methods.* Phenformin† (N-beta-phenethylformamidinyliminourea hydrochloride) was given orally (by stomach tube), subcutaneously and intraperitoneally. Tolbutamide, 1-butyl-3-p-tolylsulfonylurea (Na-Orinase‡) was administered both subcutaneously and intra-

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* Ames Company, Inc., Elkhart, Indiana.

† Generously supplied by Dr. H. S. Sadow, U.S. Vitamin & Pharmaceutical Corporation, New York, N.Y.

‡ Obtained through the courtesy of Dr. C. J. O'Donovan, The Upjohn Co., Kalamazoo, Michigan.

peritoneally. NPH* (aqueous) insulin was used by the subcutaneous route. All hamsters received single injections either at one or two sites. When compound solutions intended for gastric tube feedings exceeded 1.5 cc., they were administered in two divided doses within fifteen minutes. The animals were maintained on Purina laboratory chow with supplementary wheat germ flakes and water ad libitum.

A total of 116 *normal* adult hamsters were employed to establish maximum tolerated doses of each compound and to evaluate *drug toxicity*. Two to four animals (males and females) were used for each dose level of the drugs and each route of administration.

Evaluation of drug toxicity was difficult because many animals died of hypoglycemia before any toxic effects could be observed histologically. Clinical evidence of toxicity, however, occurred both with phenformin and tolbutamide, depending upon the route of inoculation, even before hypoglycemic shock resulted. The interval between drug administration and death, from either hypoglycemia or other causes, was timed routinely.

In *diabetic* hamsters, comparative studies were done on the relative effectiveness of the agents in reducing elevated blood sugar levels. The least toxic route of administration was chosen for *therapy*.

RESULTS

A. *Toxicity in Normal Hamsters.* When phenformin was given subcutaneously, drug induced hypoglycemia reached its maximum in about five hours. Animals given 450 mg./kg. developed reversible hypoglycemic shock; at 540 mg./kg. (LD_{50}) half, and at 630 mg./kg. (LD_{100}) all hamsters died from hypoglycemia. Clinical signs were: fast and spasmodic breathing, hyperactivity, and terminal convulsions; blood sugar levels were as low as 20 to 40 mg. per 100 ml. The majority of animals, however, died an unexplained death almost immediately after injection, usually within five to thirty minutes and before lowering of the blood sugar level. Similarly, upon *intraperitoneal* injection of phenformin, most of the animals died without overt symptoms within one to fifteen minutes; when 180 mg./kg. (LD_{50}) was given, half of the animals living beyond the first hour died from hypoglycemia between five and fifteen hours. Little toxicity was encountered upon *oral* administration, except for one death occurring within five minutes at 180 mg./kg. Irreversible hypoglycemia was induced via this route in approximately half of the animals with 1,620 mg./kg. (LD_{50}).

Tolbutamide given *subcutaneously* produced a hypoglycemic effect within three to four hours. At a dose of 178.5 mg./kg. (LD_{50}), half of the animals died from hypoglycemia between five and sixteen hours.

When tolbutamide was administered *intraperitoneally*, deaths from hypoglycemia occurred in half of the animals between five and sixteen hours at 178.5 mg./kg. (LD_{50}). Early and unexplained deaths, occurring within five to thirty minutes without hypoglycemia, resulted from doses of 2,412 mg./kg. to 4,824 mg./kg.

NPH insulin caused hypoglycemia within five to eight hours and later, with doses ranging from 16 to as many as 88 units per hamster (average weight 32 gm.).

B. *Therapy in Diabetic Hamsters.* In mildly diabetic hamsters (blood glucose between about 150 to 300 mg. per 100 ml.) it was possible to reduce elevated blood sugar levels to normal by appropriate doses of each of the drugs. The correct dosage was reached by gradual increases in daily amount. The starting dose depended upon the degree of diabetes, but was roughly one which lowered the blood glucose level in both healthy animals to about half normal. Normal blood sugar levels in severely diabetic animals, excreting 10 to 15 per cent or more urinary sugar per diem, could be maintained only with NPH insulin; both phenformin and tolbutamide were ineffective. In a few instances, diabetic hamsters died soon after the administration of any of the three compounds, before a normal sugar level could be reached. Some animals required increasingly higher drug levels over a period of weeks, indicative either of resistance or progressive worsening of the condition.

Since some deaths were attributed to the daily handling and transfer of animals from one cage to another for breeding purposes, it was decided to supply phenformin or tolbutamide in the drinking water. This proved helpful and time-saving as the number of diabetic hamsters increased. Only animals totally dependent upon insulin were injected daily. Phenformin was given at 30 mg./100 cc., and tolbutamide at 134 mg./100 cc. in water bottles. The bottles were renewed daily. The dilutions were more or less arbitrary, but daily water intake and relative effectiveness of the compounds were taken into consideration. Most animals maintained themselves at moderately hyperglycemic levels, the mortality rate was considerably reduced from about 30 per cent to 5 per cent over a half-year period, and fertility and the number of complete pregnancies increased. Quantitative data on survival are not yet at hand. In the course of therapy it became obvious that the sensitivity of diabetic animals to hypoglycemic drugs was greater than that of normal animals; comparatively less compound was required to lower elevated sugar levels than to induce hypoglycemia in normal hamsters. For instance, 2 to

*Eli Lilly and Company, Indianapolis, Indiana.

40 units of NPH insulin per hamster (30-33 gm.) would (often when 70 to 100 U. or more was required) reduce elevated blood sugar levels to normal, even in severe diabetics; in order to induce hypoglycemic shock in normal controls 40 to 90 U. were required.

DISCUSSION

In mild to moderately severe cases of diabetes, there was an almost linear relationship between reduction of the urine sugar excretion and dosage of NPH insulin. In severely diabetic hamsters, insulin was the only effective drug. It is, therefore, doubted, contrary to the reports of others, that phenformin can act in *complete* absence of insulin.[5] It was estimated that phenformin replaced roughly one fourth of the insulin required for adequate therapy in mild to severely diabetic hamsters. Diabetic animals were, within limits, comparatively more sensitive to the hypoglycemic action of phenformin than normal animals.

Although LD_{50}'s had not been determined in other animal species at the time of Ungar's report in 1957,[5] semiquantitative data on the action of phenformin on the blood sugar in various normal animals would suggest that the species most sensitive to subcutaneous inoculation of phenformin is the rhesus monkey. The species most resistant was found to be the rat. Normal Chinese hamsters were approximately ten times more resistant than the rat and fifty times more resistant than the rhesus monkeys to phenformin given subcutaneously. This observation was made by comparing the LD_{100}'s of our normal hamsters with those determined and published for other animals.[5,6] The subcutaneous administration, however, proved somewhat erratic, since many animals died almost immediately following injection, or within fifteen minutes thereafter. The oral route of administration caused fewest toxic deaths.

Tolbutamide, administered subcutaneously and intraperitoneally, was about as effective in normal hamsters as was oral or intravenous administration in normal rats.[6]

The doses of subcutaneous NPH insulin (about 500 U./kg.) required to lower the blood sugar levels to about half of normal were larger for normal hamsters than for any other species.

From the data presented in this report and those available in the literature it may be extrapolated that on a weight basis, normal hamsters were approximately ten to twelve times more resistant to tolbutamide, eight times to phenformin, and about 150 times to NPH insulin, than man. The exact reasons for these and the underlying metabolic species-differences are obscure.

SUMMARY

Methods for successful maintenance of Chinese hamsters suffering from a spontaneous hereditary diabetes are described. Therapy with either phenformin, tolbutamide and/or NPH insulin increased fertility, improved the number of successful pregnancies, and reduced the losses of liveborn animals. Toxicity studies on normal hamsters proved them to be more resistant than other species, including man, to all three agents. Spontaneous hereditary diabetes mellitus in Chinese hamsters provides an excellent tool for the screening and study of potentially hypoglycemic agents.

SUMMARIO IN INTERLINGUA

Spontanee Diabete Mellite Hereditari in Hamsters Chinese (Cricetulus griseus).

III. Le Mantenentia de un Colonia de Hamsters Diabetic con le Adjuta de un Therapia Hypoglycemic

Es describite provate methodos pro le mantenentia de hamsters chinese con spontanee diabete hereditari. Therapia con phenformina o tolbutamida e/o insulina NPH augmentava le fertilitate, augmentava le numero del pregnantias successose, e reduceva le perditas subsequente de vivenatos. Studios de toxicitate in hamsters normal demonstrava que illos es plus resistente a omne le tres agentes que altere species, incluse le homine.

ACKNOWLEDGMENT

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The Blood Ketone and Plasma Free Fatty Acid Concentration in Diabetic and Normal Subjects

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The overt manifestations of ketosis in uncontrolled diabetes mellitus have been recognized for years as a serious disturbance in fat metabolism. However, little attention has been given to the small and subtle changes in ketonemia in patients with so-called "controlled diabetes." In 1947 Briggs demonstrated elevated blood ketone levels unrelated to blood glucose or urinary acetone concentrations in a group of loosely controlled diabetic patients and suggested that ketonemia was probably the best indication of control.[1] In a series of papers dealing chiefly with psychosomatic phenomena, Hinkle and his colleagues pointed out that ketonemia could fluctuate amazingly without relation to blood glucose concentration following emotional distress.[2-5] Henderson et al. found that controlled diabetics had a greater amount of acetone in their breath than nondiabetics.[6]

The purpose of the present study was to examine and delineate the chief implication of the earlier work—namely that ketone metabolism is often abnormal in diabetics controlled according to currently employed criteria. In addition, observations were made on the plasma concentrations of free fatty acids (FFA). Since these latter substances probably represent the main substrate for the formation of acetoacetic and beta hydroxybutyric acids in the liver, at least in extensive ketosis,[7] it seemed pertinent to relate the circulating concentrations of FFA to those of ketones.

MATERIALS AND METHODS

Case Material. The nondiabetic group consisted of male laboratory personnel, medical students and hospital patients free of endocrine disorder who were admitted for minor elective surgical procedures. The subjects ranged in age from twenty to seventy-nine years and in weight ratio (actual weight/ideal weight) from 0.76 to 1.53. All received a regular diet, and were without recent weight loss. Inasmuch as no difference was found between the results of the hospital patients and the personnel or students, all were grouped together as the control nondiabetic group. Diabetes was excluded on the basis of routine clinical evaluation and a normal fasting blood glucose level. All subjects had a normal plasma creatinine concentration. Fasting blood samples were obtained in the morning after an approximately fourteen-hour overnight fast. In most instances the FFA levels were drawn at a later date from individuals who had been subjects for the ketone study. The nonfasting samples were obtained from individuals of the fasting group approximately three and one half hours after a regular meal on a different day from that on which the fasting samples had been drawn. The nonfasting FFA and ketone samples were obtained concurrently.

The patients with diabetes were a group of male Veterans Administration inpatients and outpatients. They ranged in age from eighteen to seventy-nine years and in weight ratio from 0.66 to 1.50. The inpatients, who had been hospitalized for complications or regulation of diabetes, were studied after they had been stabilized and were near the end of their hospital period. The over-all control of the disease was considered grossly adequate in that the patients were free of the usual symptoms of uncontrolled diabetes and that currently accepted methods had been employed to minimize hyperglycemia, hypoglycemia and glycosuria. The patients had previously been instructed in the use of diabetic diets (American Diabetes Association—The American Dietetic Association exchange system), the adherence to which varied among the patients. Many of the subjects had chronic complications such as vascular disease, neuropathy, retinopathy, or proteinuria. No patient with extensive renal disease was included, and the plasma creatinine level was normal at the time the patients were studied. The timing of the fasting and nonfasting blood samples was the same as for the nondiabetic subjects. Ketone levels were always determined concurrently with FFA levels but the converse was not true. Consequently the data include more ketone than FFA values. The nonfasting group was composed of patients in the fasting group as well as others. In regard to the maintenance

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therapy for the fasting patients, of those in whom ketone levels were determined, eighty-seven were taking insulin, 10 to 100 units daily, three tolbutamide and fifteen were managed with diet alone; of those in whom FFA levels were measured, forty-six were taking insulin, 10 to 100 units daily, three tolbutamide and five were on diet alone. Of the nonfasting patients, in whom both ketone and FFA levels were determined, thirty-three were taking insulin, 10 to 100 units daily, three tolbutamide and four were managed with diet alone. No attempt has been made in this paper to segregate the data according to any specific complication.

Chemical Methods. The total venous blood ketones were determined according to a modification[9] of the Greenberg and Lester method.[8] In addition, silicone stopcock grease and improved clamps were employed on the glass-stoppered tubes during the heating stages. Average recoveries of acetone, acetoacetic acid and beta hydroxybutyric acid (as the sodium salt) through the entire procedure were 101, 102 and 91 per cent respectively. Glucose in high concentration increases the intensity of the final 2,4 dinitrophenylhydrazine color reaction. Thus possible interference by glucose in the routine ketone procedure was investigated extensively by adding increasing amounts of reagent grade glucose to water, aqueous ketone standards and heparinized blood prior to the ketone analysis. The interference by endogenous glucose was examined by determining ketone concentration from blood samples at different filtrate dilutions. The results indicated that there was no significant interference by endogenous glucose in the ketone values performed in the standard procedure up to a blood glucose concentration of 300 mg. per 100 ml. For blood samples in the study which contained glucose in amounts higher than 300 mg. per 100 ml. the filtrates were further diluted, or, in a few cases in which there was no more filtrate available, a standard correction factor predetermined according to the glucose level was employed. To our knowledge there is nothing else in blood from patients with diabetes which would interfere in the ketone method although this has not been proved conclusively by actually isolating the ketone bodies. The mean error of reproducibility for the ketone procedure was 7 per cent.

The heparinized venous blood samples for FFA analysis were cooled immediately after blood letting and the cooling was maintained during centrifugation and until the plasma was drawn off and frozen. The FFA were analyzed according to the method of Bierman et al.[10] and are expressed as μM./L. The error of reproducibility of the analysis was less than 5 per cent. Blood glucose was analyzed according to the Somogyi-Nelson procedure.[11] Urinary "ketones" were determined by means of the Acetest tablet, a nitroprusside reaction which measures mainly acetoacetic acid. A trace positive corresponds to a concentration of acetoacetic acid of 5 mg. per 100 ml. (490 μM./L.); a moderate positive to 30 mg. per 100 ml. (2,937 μM./L.); and a strong positive to 80 mg. per 100 ml. (7,832 μM./L.).[12] The tests were performed according to the manufacturer's instructions. The color change was usually viewed by two observers to find as many positives as possible. Occasionally it was difficult to decide whether a specimen was negative or slightly positive and these were recorded as questionable.

Statistical evaluation included the t-test, analysis of variance and the coefficient of correlation.[13] Only initial levels on patients were included in statistical analyses. Subsequent levels are included in scattergrams.

RESULTS

1. *The concentrations of blood ketones and plasma FFA in nondiabetic and diabetic subjects (initial levels) table 1.* In the nondiabetic subjects the mean concentration of blood ketones in those who were fasting was 143 μM./L. and in those who were not fasting 107 μM./L., which was probably significantly lower. In the diabetic patients the mean fasting blood ketone concentration was 402 μM./L. and nonfasting 257 μM./L. Both of these values were significantly elevated above those observed in the corresponding state in the nondiabetic subjects. However, the decrease among the diabetics after eating was not statistically significant, apparently because of the wide scatter in values.

The mean FFA concentrations were similar in the fasting and nonfasting nondiabetic groups, 554 and 570 μM./L. respectively. In the diabetics the mean fasting concentration of 775 μM./L. was greater than that observed in the fasting nondiabetics whereas the mean nonfasting FFA level in the diabetics was not significantly different from that in the nondiabetics. The postprandial decrement among the diabetics was probably significant.

The values were subclassified within groups according to age and weight and tested for mean differences by analysis of variance. In neither the normal nor diabetic, fasting nor nonfasting groups, could any significant influence of age or weight be demonstrated. However, it is worth noting that in the diabetics there was a tendency for the nonfasting ketone levels to be lower with increasing weight. The corresponding mean FFA levels determined on samples drawn concurrently in the same patients did not suggest this as clearly.

2. *The relation of the blood ketone and plasma FFA concentrations to those of blood glucose simultaneously*

TABLE 1

The levels of blood ketones and plasma FFA in nondiabetic and diabetic subjects*

Group	Blood ketones μM./L. Fasting	Nonfasting	P	Plasma FFA μM./L. Fasting	Nonfasting	P
Nondiabetic	143 (94)† ±69	107 (19) ±70	<0.05	554 (61) ±169	570 (19) ±164	>0.5
Diabetic	402 (105) ±555	257 (39) ±174	>0.1	775 (54) ±318	630 (39) ±294	<0.05
P	<0.01	<0.01		<0.01	>0.1	

* Mean ± Standard Deviation; data based on levels from the subjects' initial blood samples for the respective determination.

† The numbers in parentheses refer to the number in each respective group.

obtained, in the diabetic group. The mean values, from the patients' initial blood samples, along with the statistical analyses, are presented in table 2. The total values, including those from repeated blood samples obtained on different days from the same patients, are plotted as scattergrams in figures 1 through 4. In the figures the values of the means plus two standard deviations for the corresponding nondiabetic groups are shown for comparison. The statistical evaluations of the initial values (table 2) appear to correlate with the total values as illustrated in the scattergrams.

Though there is considerable scatter, it is apparent that the fasting ketone concentrations in diabetics increased with those of glucose and also ranged significant-

TABLE 2

Blood ketones and plasma FFA levels (μM./L.) in male diabetics,* grouped according to blood glucose concentrations

Analysis	Fasting patients Blood glucose ranges mg. per 100 ml. 30-59	60-99	100-124	125-149	150-199	200-299	300-475
Ketones							
Mean	256	201	358	365	329	692	939
S.D.±	156	81	274	188	178	1,140	896
N	6	17	13	16	30	19	4
P vs. nondiabetic	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
FFA							
Mean	841	577	906	750	779	763	1,204
S.D.±	138	207	417	260	251	317	739
N	3	8	3	8	15	14	3
P vs. nondiabetic	<0.01	>0.5	<0.01	<0.01	<0.01	<0.01	<0.01

	Nonfasting patients Blood glucose ranges mg. per 100 ml. 60-99	100-149	150-199	200-299	300-475
Ketones					
Mean	176	162	194	386	435
S.D.±	82	111	122	188	214
N	10	7	8	10	4
P vs. nondiabetic	<0.05	>0.1	<0.05	<0.01	<0.01
P vs. fasting diabetic	>0.1	<0.05	>0.05	>0.1	>0.1
FFA					
Mean	650	544	558	675	760
S.D.±	387	225	196	326	268
N	10	7	8	10	4
P vs. nondiabetic	>0.1	>0.5	>0.1	>0.1	<0.01
P vs. fasting diabetic	**>0.5**	>0.1	<0.05	>0.5	>0.1

* Data based on levels from the subjects' initial blood samples for the respective determination and represents a subclassification of table 1.

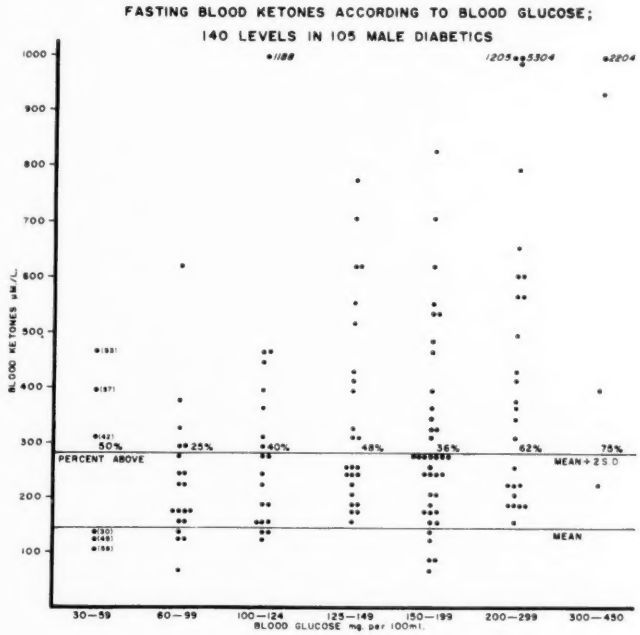

FIG. 1. The lower horizontal line represents the corresponding nondiabetic mean concentration and the upper horizontal line the mean plus two standard deviations.

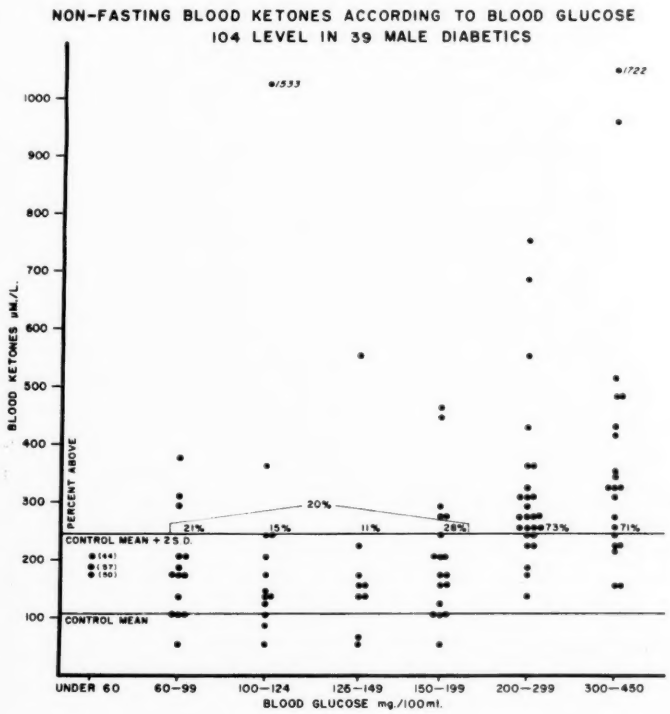

FIG. 2. The lower horizontal line represents the corresponding nondiabetic mean concentration and the upper horizontal line the mean plus two standard deviations.

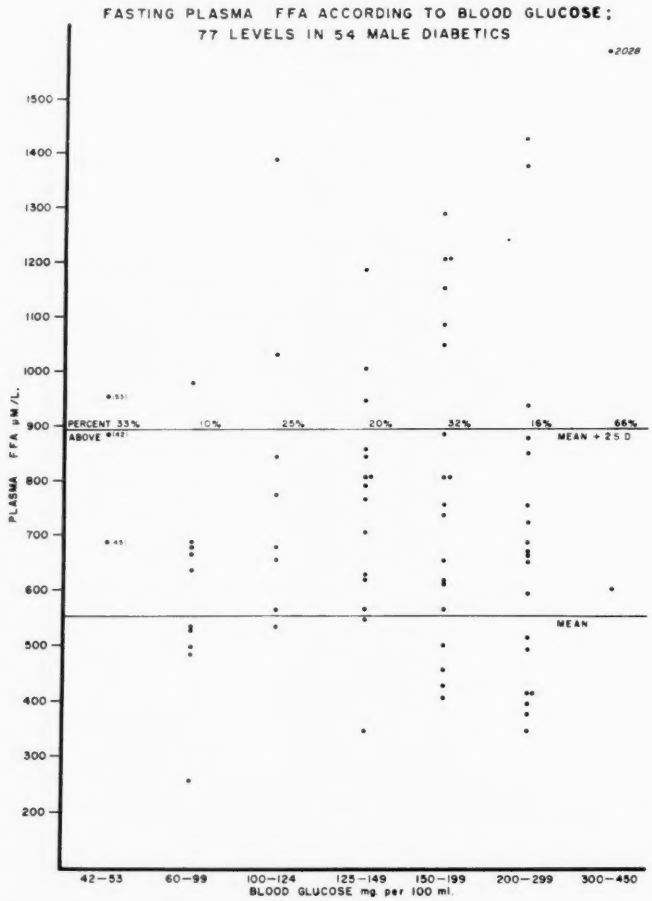

FIG. 3. The lower horizontal line represents the corresponding nondiabetic mean concentration and the upper horizontal line the mean plus two standard deviations.

ly above the nondiabetic mean at each glucose division, including the strictly normal glucose range, 60 to 99 mg. per 100 ml. Of the six fasting ketone levels associated with hypoglycemia (blood glucose 30 to 59 mg. per 100 ml.), three were elevated above the nondiabetic mean plus two standard deviations. The nonfasting ketone levels also appeared to increase with glucose concentration and ranged significantly above the nondiabetic mean at all glucose divisions except the approximately normal postprandial glucose range, 100 to 149 mg. per 100 ml. The nonfasting levels tended to be lower than the fasting values for each glucose range but the difference was only statistically significant at the glucose range of 100 to 149 mg. per 100 ml.

There did not appear to be the correlation with the fasting FFA and glucose levels that was apparent with the ketone concentrations. When the concentration of glucose was below 100 mg. per 100 ml. the values of fasting FFA were equivalent to those of the nondiabetics. The concentrations of FFA ranged above those of nondiabetics when the blood glucose concentration was between 100 and 124 mg. per 100 ml. but failed to increase further with rising concentrations of glucose. Except for a few instances the values for nonfasting FFA differed little from those of nondiabetics until the blood glucose concentration exceeded 200 mg. per 100 ml. when there appeared to be some increase. The nonfasting levels tended to be less than the fasting levels at a blood glucose concentration of 100 to 200 mg. per 100 ml.

3. *The relation of the blood ketone concentrations to those of plasma FFA.* There was an insufficient number of paired ketone and FFA values in the fasting nondiabetics to determine a relationship. The correlation coefficient between the paired nonfasting nondiabetic ketones and FFA was not significant.

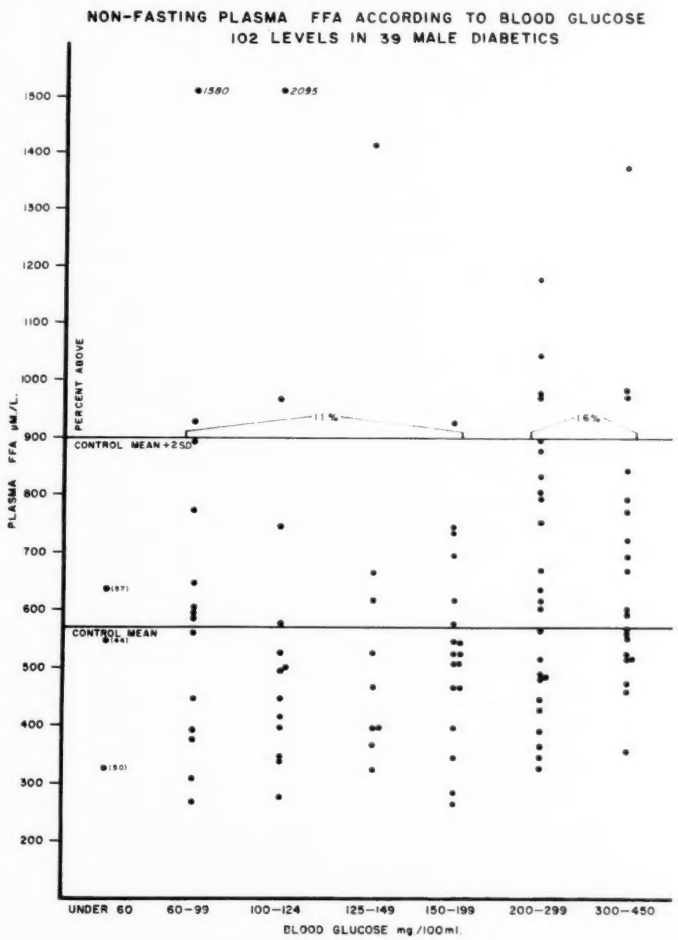

FIG. 4. The lower horizontal line represents the corresponding nondiabetic mean concentration and the upper horizontal line the mean plus two standard deviations.

In the diabetics the correlation coefficients between initial paired ketone and FFA levels, fasting and nonfasting, were 0.50 and 0.68, respectively, both statistically significant ($P<0.01$).

The total paired levels in samples obtained simultaneously from diabetics, fasting and nonfasting and including those from repeated blood letting, are shown in figure 5. The general positive correlation between the ketones and FFA is depicted. However, it is apparent from the graph that the ketones were frequently elevated above the nondiabetic range in the presence of a normal FFA concentration. The frequency with which this occurred is indicated in the tabular insert. In fifty-eight instances the ketone concentration was above normal when the FFA concentration was normal, whereas the converse was observed in only seven instances. The relationship of high FFA levels to normal or slightly elevated ketones may represent significant individual differences. One obese patient repeatedly demonstrated these findings.

4. *The concentrations of blood ketones and plasma FFA in stable and unstable diabetics.* A. Since the majority of the patients studied were taking insulin, the data of those who were managed with diet alone or diet in conjunction with tolbutamide were examined separately. There were fourteen such patients with initial fasting samples who had negative to two-plus glycosuria and a negative Acetest. The mean blood glucose concentration was 141 mg. per 100 ml. (range 78 to 197). The mean blood ketone concentration of this group, 242 μM./L., was significantly elevated above the fasting nondiabetic group ($P<0.01$). Two of five FFA levels were elevated above the normal range. Only a few initial samples were obtained in nonfasting patients not taking

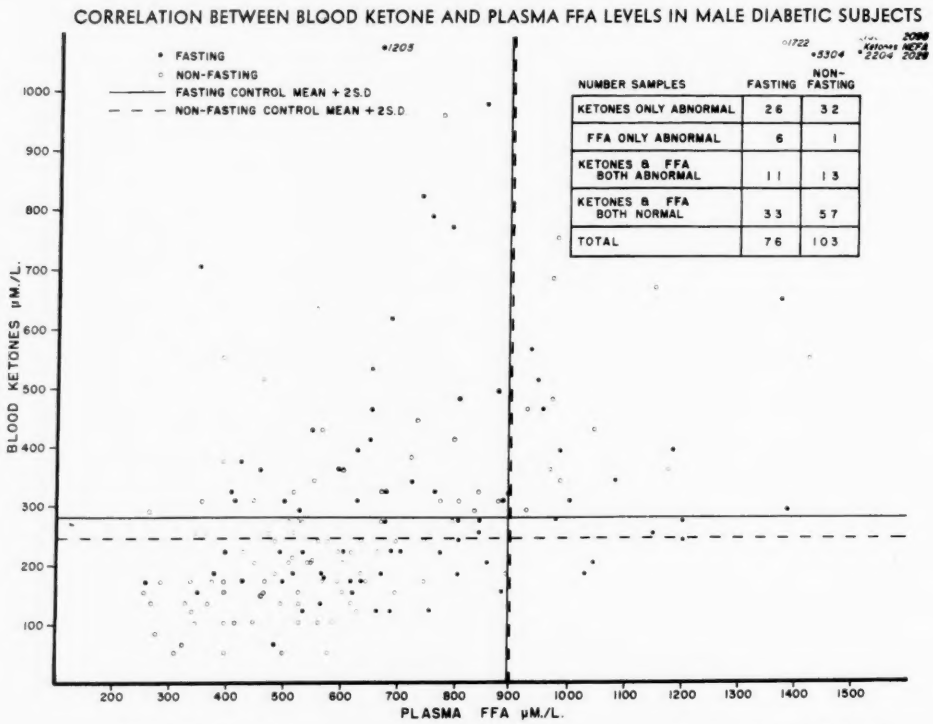

FIG. 5. The control means plus two standard deviations refer to the respective nondiabetic groups.

insulin, and the ketones and FFA levels were in the normal range.

B. In figure 6 are plotted the mean concentrations of blood glucose, ketones, and plasma FFA observed during prolonged morning fasting in two groups of diabetic patients, one representing the older stable population, and the other the younger unstable population.

The fasting glucose concentrations were higher in the younger group, a not unexpected finding in view of the difficulty of control in this group. However, the observation of similar curves of FFA and ketone concentrations was unexpected. Because of the common occurrence of fasting ketonuria in the unstable diabetics, it was expected that more elevated ketone and FFA concentrations would be found in these patients. Another point of interest is the similarity in configuration of the curves between the two groups. It is possible that both ketone curves fundamentally were linear throughout in ascent and the initially elevated values were influenced by the anticipatory stress of initial venipuncture.

Shown also in figure 6 are the values observed in two patients with sustained hypoglycemia during the four hours of the test. The concentrations of both ketones and FFA rose over the four hours in both patients though the changes in one patient were particularly marked, and the elevations were considerably greater than the mean levels in the two hyperglycemic groups.

5. *The relation of the urine Acetest to the blood ketone concentration.* Blood samples for ketone analysis and urine samples for Acetest were obtained at approximately the same time in 216 instances (108 fasting and 108 nonfasting) from ninety-four diabetic patients. The findings were plotted in figure 7.

Seventeen of nineteen samples with a trace positive urine Acetest and all sixteen samples with moderately or strongly positive Acetest were associated with blood ketone levels exceeding the mean plus two standard deviations of normal. However, fifty-five of 172 samples (32 per cent) with a negative urine Acetest were associated with blood ketone concentrations exceeding this normal range. From this it would appear that though the urine Acetest may be moderately sensitive, frequently it will not reflect elevations of total blood ketone concentrations above the normal range.

DISCUSSION

In the present study there did not appear to be any significant relation of blood ketone and plasma FFA concentration to age or weight. Similarly, a lack of effect

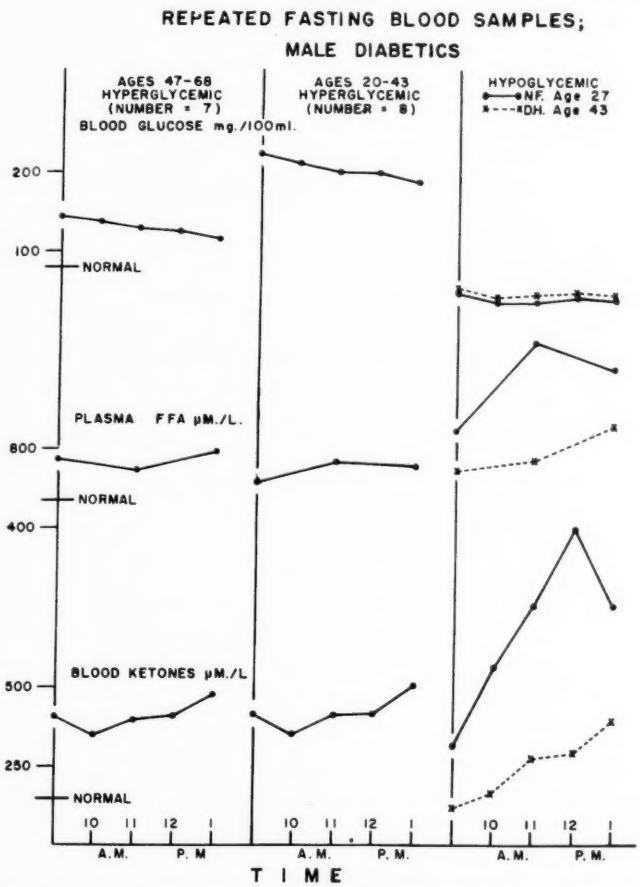

FIG. 6. The patients in the hyperglycemic age group, forty-seven to sixty-eight, are representative of stable diabetics and those in the hyperglycemic age group, twenty to forty-three, are representative of unstable diabetics. All the patients in the latter group were taking insulin, 20 to 50 units daily, whereas in the former group, four patients were taking insulin, 10 to 30 units daily, and the remaining three took no insulin. The hypoglycemic patients took 65 and 80 units daily, respectively. The horizontal bars depict the nondiabetic mean levels.

by age on ketones was observed by Henderson et al. in their study of the concentration of acetone of the breath.[9] However, ketosis has been reported to be decreased in obese persons, as compared to nonobese, during prolonged fasting.[14] The findings in the present study are not comparable in that the fasting extended overnight only. In the diabetics, there was a tendency albeit not statistically significant, for the ketone concentrations to decrease with the degree of overweight. The failure to find elevation of the FFA concentration in the nondiabetic overweight subjects was surprising in view of Dole's earlier observations to this effect.[15] The apparent difference may relate to the fact that his subjects were more obese and suffered from a variety of illnesses. One obese diabetic in this study persistently had a high FFA concentration, with only a slight elevation in ketones, findings similar to those reported by Gordon.[16]

The observation of a blood ketone concentration lower in fed than fasted nondiabetic subjects was expected in view of the known sharp inhibitory effect of carbohydrate administration on hepatic ketogenesis. The fact that this effect was not confirmed by statistical analysis in the diabetics appears to be related, at least in part, to the wide scatter of values in the fasted and to a lesser extent in the nonfasted group. In contrast to the findings relative to the ketones, feeding appeared to have no effect on the plasma FFA in the normals and a significant lowering effect in the diabetics. Dole did observe a fall in the FFA concentration after eating in nondiabetics.[18] However, his samples were obtained one hour after eating while in the current study the samples were drawn, on the average, three and one-half hours after the previous meal. The nadir in plasma FFA concentration normally occurs in one to two hours following pure carbohydrate ingestion[15,17] while in the diabetic it seems to be prolonged.[10] Such a difference in time of effect of carbohydrate between the normal and diabetic groups probably influenced the nonfasting FFA results within each group. This may also account for the finding that the nonfasting FFA of diabetics were not significantly ele-

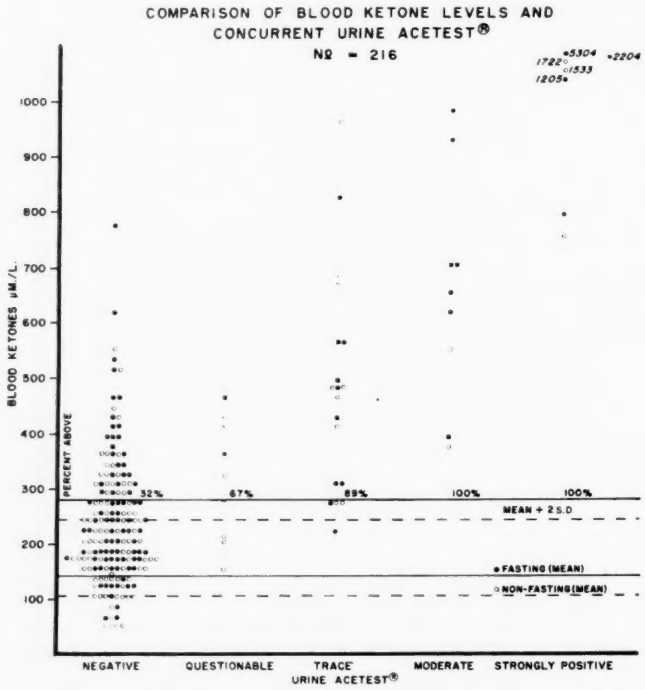

FIG. 7. The horizontal lines refer to the respective nondiabetic (control) groups.

vated above those of the nondiabetic. Pure fat feeding produces a variable rise in FFA levels in approximately four hours in the normal individual[16,17-19] but its effect in the diabetic is not known.

It is evident from the study that the average concentrations of blood ketones and plasma FFA in the fasting state were in excess of normal, findings which are in general agreement with those of Briggs,[1] and Bierman et al.,[20] respectively. The observation of hyperketonemia and increased FFA concentration in the stable diabetics and those not receiving insulin would rule against the possibility that the fasting hyperketonemia in the insulin treated diabetics was produced solely by nocturnal insulin hypoglycemia prior to blood letting. To a certain extent the elevations are attributable to the fact that the patients were actually not well controlled as indicated by the often present hyperglycemia and the occasional ketonuria seen so frequently in large clinic populations. However, particular emphasis must be placed on the finding of persistent slight hyperketonemia when the blood sugar concentrations were within the strictly normal range. This would suggest that all parameters of control in the diabetic may never be satisfactorily achieved when hyperglycemia is relieved. These substances along with the serum triglycerides[20-22] may be a more accurate index than glucose of labile intracellular abnormalities. With only slight hyperglycemia, up to 150 mg. per 100 ml., in the fasting state both the ketones and FFA were significantly elevated. Conversely at glucose levels up to 200 mg. per 100 ml. in the postprandial diabetic these substances tended to be normal, suggesting that the intracellular abnormalities were temporarily corrected despite hyperglycemia.

The studies of Hinkle et al. revealed elevated ketone concentrations in diabetic subjects undergoing emotional stress.[2-5] In some instances these concentrations, in relation to blood glucose levels were considerably above those observed in the present study, probably because emotional stimulation was avoided as far as possible in the latter. Hinkle and his colleagues suggested that ketonemia would not be a useful index in the management of diabetics because the values were either disproportionally high following emotional conflicts or were normal. Slight elevations in many patients reported here are not consistent with this view. Furthermore, the disproportionate hyperketonemia in diabetics under psychic stress could be advantageous in evaluating control by virtue of its very sensitivity.

The finding of a significant positive correlation between the ketone and FFA values would be expected on the basis that FFA probably represent the principal hepatic substrate for ketone production.[7] Prolonged he-

patic ketogenesis is very likely dependent on increased FFA mobilization from peripheral fat depots and transport to the liver. The few patients with very high ketone levels in this study had high plasma concentrations of FFA.

It is of interest that the concentrations of blood ketones were more apt to be elevated in conjunction with normal concentrations of FFA than the converse. At least two factors tend to explain this. It can be estimated from published data that the normal fasting splanchnic ketone production would require the equivalent of only about 10 per cent of the normal fasting splanchnic FFA uptake.[6,28] Thus it seems reasonable that hepatic ketone production could be increased by enhancing the proportion of FFA directed toward ketogenesis. Secondly, and possibly more importantly, when one considers the magnitude of the turnover rate of the plasma FFA[24] it may be expected that the delivery of FFA to the liver may be increased in such degree as to cause significant ketone production with little or no change in the plasma FFA concentration. In diabetic acidosis the blood ketones may exceed 5,000 μM./L., a twentyfold increase from a high normal level of 250 μM./L., whereas the corresponding rise in FFA concentration is roughly from 700 to 2,100 μM./L., or only a threefold rise. Consequently, it would appear that the circulating blood ketone concentration is a much more sensitive index of lipid abnormality than is the plasma FFA concentration. It is possible that the opposite condition of high plasma FFA with normal or slightly elevated ketones, observed in this study in an obese diabetic, may represent a somewhat different abnormality.

An excellent correlation between a positive urine Acetest and hyperketonemia was found. Thus the validity and usefulness of a positive Acetest are illustrated. The weakness in the test was shown by the fact that one third of the patients with a negative urine Acetest had slightly elevated blood ketone levels. There are undoubtedly several reasons for this. The blood ketone method measures both beta hydroxybutyric acid and acetoacetic acid, whereas the Acetest measures principally the latter and is not as sensitive as the blood method. Timing of the sampling and alterations in renal function may also be factors.

Whether there is any connection between slightly increased ketone and FFA levels and the development of vascular complications is, of course, unknown. However, it appears likely that mild elevations in circulating ketones and FFA indicate abnormally regulated intracellular metabolic processes and as such might over a period of years be associated with degenerative complications. Certainly the control of diabetes cannot be considered physiologically normal until all such aspects are corrected.

SUMMARY

Fasting and postprandial (three and one-half hours) blood ketones and plasma free fatty acids (FFA) were measured in a large number of male nondiabetic subjects and "controlled" diabetic patients.

Compared to those of fasting nondiabetics the concentrations of ketones and FFA were greater in the fasting diabetic subjects. In the postprandial state ketone but not FFA levels were higher in diabetic than in nondiabetic subjects.

In nondiabetics, postprandial ketone concentrations were significantly less than fasting, but FFA were not different. In diabetics the reverse was true.

Ketone values increased with blood glucose concentration in diabetics and fasting levels were greater at all glucose ranges, including normal, than in nondiabetic subjects. Ketones in nonfasting diabetics with mild hyperglycemia were not different from postprandial nondiabetics. A positive relationship with glucose concentration was not as apparent with FFA. In fasting diabetics FFA were elevated at all glucose ranges except normal whereas in postprandial diabetics FFA were generally not increased above normal.

Stable diabetics often showed elevated ketones and FFA comparable to those of unstable diabetics, despite lower blood glucose concentrations in the former.

A significant positive correlation was demonstrated between ketone and FFA values, but in general ketone levels were more apt to be increased with normal FFA than the converse. A positive urinary Acetest correlated well with hyperketonemia but slightly elevated ketone levels were often found with a negative Acetest.

It is concluded that the concentration of circulating ketones and FFA are sensitive indices of slightly abnormal intracellular processes in regulated diabetics and should be given consideration in striving for strict physiologic normalcy in this disease.

SUMMARIO IN INTERLINGUA

Le Concentration de Cetonas in le Sanguine e de Libere Acidos Grasse in le Plasma de Subjectos Diabetic e Normal

Le concentrationes de cetonas del sanguine e de libere acidos grasse del plasma esseva mesurate in stato jejun e tres horas e medie postprandialmente in un grande numero de non-diabetic subjectos mascule e de patientes del mesme sexo con diabete in stato subjugate.

In stato jejun, le concentrationes del cetonas e del libere acidos grasse esseva plus grande in le subjectos

diabetic. In stato postprandial, le concentrationes del cetonas sed non illos del libere acidos grasse esseva plus grande in le subjectos diabetic.

In subjectos non-diabetic, le concentrationes postprandial de cetonas esseva significativemente plus basse que le correspondente concentrationes in stato jejun. In subjectos diabetic, le contrario esseva ver.

Le valores del cetonas cresceva con le concentration de glucosa in le sanguine del subjectos diabetic. Illos esseva plus grande que in le caso del subjectos non-diabetic, e isto valeva pro omne nivellos de glucosa, incluse le nivello normal. Le valores del cetonas in diabeticos non in stato jejun e con leve grados de hyperglycemia non differeva ab le correspondente valores in subjectos non-diabetic. Un correlation positive con le valores de glucosa in le sanguine non esseva apparente pro libere acidos grasse. In diabeticos in stato jejun, le nivellos de libere acidos grasse esseva elevate a omne nivellos de glucosa con le exception del nivello normal, durante que in diabeticos in stato postprandial le nivellos de libere acidos grasse esseva generalmente non elevate in supra del norma.

Diabeticos stabilisate monstrava frequentemente elevate nivellos de cetonas e de libere acidos grasse con valores simile a illos observate in diabeticos non-stabilisate, in despecto del plus basse concentrationes de glucosa in le sanguine del diabeticos stabilisate.

Un significative correlation positive esseva demonstrate inter le valores del cetonas e illos del libere acidos grasse, sed a generalmente parlar le nivellos del cetonas esseva plus apte a esser elevate in le presentia de nivellos normal de libere acidos grasse que inversemente. Un positive "Acetest" urinari esseva ben correlationate con hypercetonemia, sed levemente elevate nivellos del cetonas esseva frequentemente incontrate con negative tal tests.

Es concludite que le concentration de cetonas e libere acidos grasse in le circulation es sensibile indices de levemente anormal processos intracellular in patientes con diabete regulate e deberea esser prendite in consideration in le effortio de establir le plus perfecte stato de normalitate physiologic possibile in iste morbo.

ACKNOWLEDGMENT

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The Cortisone-Glucose Tolerance Test with Special Reference to the Prediction of Diabetes

Diagnosis of Prediabetes

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The term "prediabetes" includes the condition of a woman in the years before she becomes overtly diabetic, during which she is liable to give rise to over-large babies and stillbirths. She may have a diabetic family history, show glycosuria only in pregnancy, and gain weight rapidly after it. The stress of a pregnancy, an infection, or cortisone therapy may induce a "temporary" diabetic state, with apparent normalcy afterwards.[1] Probably the best evidence of prediabetes is to be found in the pancreas of the mother's stillborn infant.[2] If Rh incompatibility is excluded, a gross enlargement of the islets of Langerhans (perhaps better called the "continents" of Langerhans) appears to occur only in the presence of maternal diabetes or prediabetes. Many unexplained stillbirths may be better understood if the pancreas is carefully examined.[3]

Other persons, both men and women, who must also be considered prediabetic, are the children of parents who were both diabetic, the identical twin of a diabetic patient, and a person with a parent and a child who are both diabetic, where there is no known diabetes on the spouse's side. There are other hints of prediabetes, but these will not be considered here.

When prediabetes is suspected, attempts may be made to make the diagnosis more secure. Often the simple 50 gm. oral glucose tolerance curve may be slightly abnormal—usually with just a high two- or two-and-one-half-hour figure. Patients with this minor abnormality who have been watched have demonstrated its significance by later becoming overtly diabetic.[4]

Fajans and Conn[5] attempted to sensitize the simple oral glucose tolerance test by preliminary priming with 100-125 mg. of cortisone. By this means they were able to show that 24 per cent of subjects with a positive family history gave abnormal responses to this test, as against only 3 per cent of those without diabetes in their families. Their results did not, however, answer the question whether they were, in fact, "predicting" the appearance of future diabetes.

We thought that a more searching evaluation of this method would entail its use in more definitely prediabetic subjects, especially the groups mentioned above. West[6] has since published something on these lines, though he estimated only single blood sugar levels taken two hours after glucose. He examined four sibships with both parents diabetic, and found that 50 per cent (seven out of fourteen) of subjects gave a negative response, which was no lower a figure than he obtained in subjects with a much less intense family history. He also observed a tendency for patients over thirty years of age to give a more positive response to cortisone than younger subjects. We have attempted to enlarge on these reports, and have found some unexpected features with relation to age, pregnancy and mild diabetes.

METHOD

Subjects

The tests were performed on the following groups:

1. Normal adults under forty-five years of age without history of diabetes in their families.*

2. Normal adults forty-five years or more of age without history of diabetes in their families.

This older age group was chosen for three reasons. First, we noticed a tendency for a number of abnormal

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*The absence of known diabetes in a family is, of course, no guarantee that any given member will not become diabetic. However, diabetes is considerably more likely to occur when the family history is positive, so that it is better to omit subjects in such families from a normal control group. Similarly, we omit women who have had large babies or stillbirths, or have shown any other suggestion of the prediabetic state.

curves to appear at about this age. Second, this is the approximate menopausal age in women, beyond which diabetes becomes much more common. Third, many of the patients whom we wished to test were over forty-five, and a standard applicable in youth may not be equally valid in older subjects.

Subjects in groups 1 and 2 included medical students, doctors, laboratory workers, and outpatients attending hospital clinics for reasons which should have no effect on their metabolic status. White and colored (half-caste) subjects were used. The incidence and types of true diabetes appear about the same in the two racial groups, but pure Bantu subjects were not included.

3. Normal pregnant women, without diabetes in their families and with normal obstetric histories, tested at various stages of pregnancy, and up to the fifth post-partum day.

Subjects in this group were doctors, doctors' wives, laboratory workers and volunteers from an antenatal clinic, chosen without reference to age or parity.

4. Women believed to be prediabetic on the basis of strong obstetrical evidence, including, in some cases, the finding of enlarged islets of a stillborn infant.

5. Subjects whose family history indicated that they should be considered prediabetic (e.g., both parents diabetic).

6. Mild diabetics (none taking insulin), including some whose diabetes was in remission following dietary restriction and weight loss.

The glucose tolerance test

All subjects ate diets high in carbohydrate for at least a week before the tests. Fifty grams of glucose were taken orally, and blood sugar readings done half-hourly for two and one-half hours. Capillary blood was used, and estimations done by Hagedorn's modification of the Hagedorn-Jensen method.[7] The test augmented by cortisone was performed the next day, or not more than one week later. Cortisone acetate was given orally in doses of 50 mg. (or 62.5 mg. if the subject was over 160 lb. in weight) eight and a half and two hours before the glucose was ingested.

Interpretation and presentation

Our criteria for abnormality of the glucose tolerance test as performed by us have been discussed in detail elsewhere.[7] In the ordinary, unprimed tolerance test we believe that figures above 120 mg. per 100 ml. fasting, 200 mg. at one hour, and 140 mg. at two hours are abnormal. If all three are exceeded the curve is considered indicative of diabetes. It is probably diabetic if only two are exceeded. A figure of 140 mg. at two hours with the other readings not abnormal, is considered suspicious of prediabetes and bears repetition and follow-up.

Fajans and Conn[6] calculated their normal limits for the augmented test to be 160 mg. at one hour and 140 mg. at two hours. They used a larger load of glucose, venous blood and a "true" blood sugar method, so that our comparable figures would presumably be 20-40 mg. above theirs. On this basis, values of 200 mg. at one hour and 180 mg. at two hours would indicate the boundaries of the abnormal zone, while a curve below 180 mg. at one hour and 160 mg. at two hours would be presumptively normal.

A statistical evaluation of results in our normal subjects below forty-five years of age (as described below) indicated a slightly different boundary zone, in which 205 mg. is the upper limit at one hour and 155 mg. at two hours, while under 185 mg. at one hour and 135 mg. at two hours are presumptively normal. This intermediate or "doubtful" boundary zone is indicated on our figures as a darkened parallelogram (figure 1).

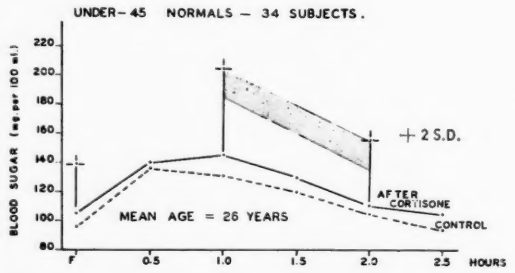


FIG. 1. Mean curves of ordinary (control) and cortisone-glucose tolerance tests. Plus two standard deviations are shown for the fasting, one- and two-hour figures of the latter curves. The shaded area, "doubtful zone," is obtained from these figures, above which definite abnormality is accepted.

We do not know whether a better criterion of abnormality might not be the amount of change at each comparable level of the two curves in the same individual, rather than the absolute levels reached in the cortisone test. In our young control group, two standard deviations from the mean one-hour level on the cortisone tolerance curve was 60 mg., and at the two-hour level it was 55 mg. We therefore accept a result of the cortisone test as being definitely positive only when the curve lies entirely above the borderline parallelogram and when at least one reading is 50 mg. or more above the corresponding reading on the basic glucose tolerance curve.

RESULTS

Control adult group, under forty-five years (thirty-six subjects)

Figure 1 illustrates the mean pre- and post-cortisone glucose tolerance curves in these subjects (mean age = twenty-six years, both sexes included). The mean one-hour figure was elevated by the cortisone 14 mg. per 100 ml. and the two-hour figure only 6 mg. Twice the standard deviation from the mean raises the acceptable upper limit at one hour to 205 mg. per 100 ml. and at two hours to 156 mg., as indicated in figure 1. In only one subject was the one-hour figure above 205 mg. (215 mg.) and in none was the two-hour figure above 155 mg. In the ordinary control glucose tolerance test, the two-hour figure exceeded 140 mg. in three cases, but in none of these was the cortisone test abnormal. In two cases the cortisone test at one hour gave a figure more than 50 mg. above the control test.

Control group forty-five years of age and over (thirty-four subjects including ten males), mean age 55.5 years including ten males

In this group we found, as have others before us, that the standard mean glucose tolerance curve was higher than in the younger group (figure 2). The difference at one hour was 31.5 mg. per 100 ml., which is highly significant ($p<.01$). On the criteria which we have accepted for the diagnosis of diabetes, four of this group would be classed as definitely diabetic, while in six the curve was abnormal (usually too high at two hours). That is, 24 per cent were apparently abnormal.

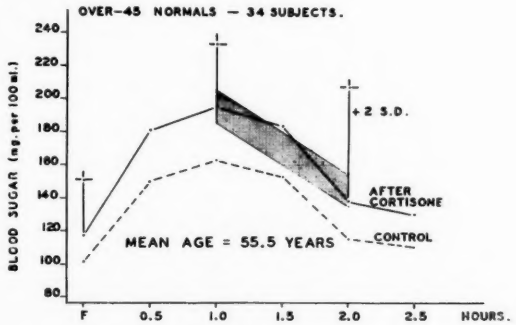


FIG. 2. Similar to figure 1. Note the higher control and cortisone curves, and also the much higher figures for plus two standard deviations.

Cortisone caused an increase of 32.5 mg. per 100 ml. in the mean one-hour figure and of 23 mg. in the two-hour figure. The mean one- and two-hour levels were both significantly higher than the corresponding figures in the under forty-five group ($p<.01$ for each). In twelve subjects a one-hour level of 205 mg. was exceeded, and in seven the two-hour figure was over 166 mg. Over one third of this group, therefore, had abnormal augmented tests, on the basis of criteria derived from the younger group. Even the mean cortisone curve is suspiciously abnormal. However, those subjects who showed abnormalities in their standard tests did not necessarily yield abnormal cortisone tests. Thus, five out of eight with two-hour figures over 140 mg. on standard testing were over 160 mg. on the augmented test.

Normal pregnant women (forty subjects, mean age 26.5 years)

Figure 3 shows the mean curves of this group. While the mean control curve was no higher than that of the young control group, the mean rise after cortisone was significantly greater ($p<.01$ for both one- and two-hour levels). In the standard test, the highest one-hour figure was 180 mg. and the highest at two hours was 135 mg. After cortisone, 205 mg. was exceeded twelve times at one hour, and 155 mg. was exceeded eleven times at two hours.

In some of these pregnant women the whole curve was enormously elevated by cortisone, as shown in figure 4. In none of them was there the slightest suspicion of prediabetes. In two it was possible to repeat the tests after delivery and in each case it had then become entirely normal (figure 4).

Prediabetic women

There were seventeen women whom we believed to be prediabetic on strong evidence. Several had glucose tolerance curves of prediabetic type, as discussed elsewhere.[7] This was the group above all others in which we hoped that the augmented tolerance test might be helpful. The mean curves are illustrated in figure 5, and it is plain that the response to cortisone was not, in general, very conclusive. In some individuals there was little or no augmentation of the curve, but in four it appeared to be helpful in confirming the diagnosis of prediabetes. All of the latter were pregnant at the time of testing. For example, in A.O. (figure 6), the control curve was quite normal, but cortisone raised the one-hour figure by more than 80 mg. per 100 ml. In C.M. (figure 7) a grossly abnormal cortisone curve appeared to confirm the prediabetic type of ordinary tolerance curve, and final confirmation appeared some months later, when overt diabetes developed.

In the case of M.L. (figure 8) cortisone augmentation was not necessary for diagnosis, since the underlying

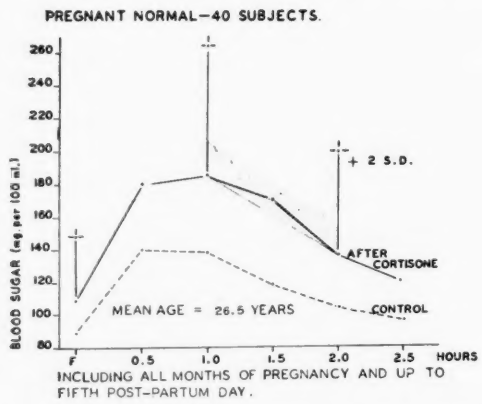


FIG. 3. Similar to figure 1. Note low control curves, but higher mean cortisone curves, and high figures for plus two standard deviations.

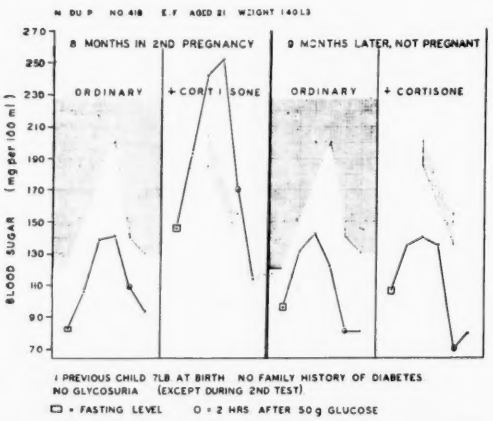


FIG. 4. Successive glucose tolerance curves; each dot represents a half hourly reading. The shaded areas in the first and third curves represent the abnormal zone in our interpretation of the ordinary glucose tolerance curve. Note unexpectedly high cortisone curve during pregnancy, with normal curve later.

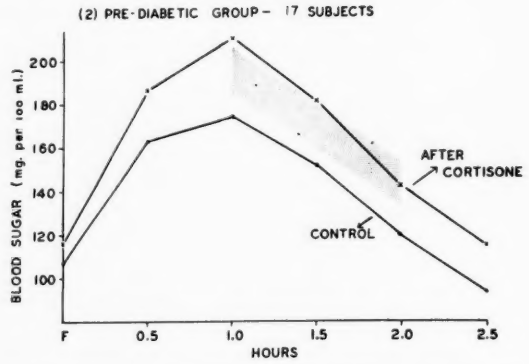


FIG. 5. Members in this group were all diagnosed as prediabetic on very strong grounds; always obstetrical and often with family history also. Note raised mean control curve. The mean cortisone curve is just abnormal, but not strikingly so.

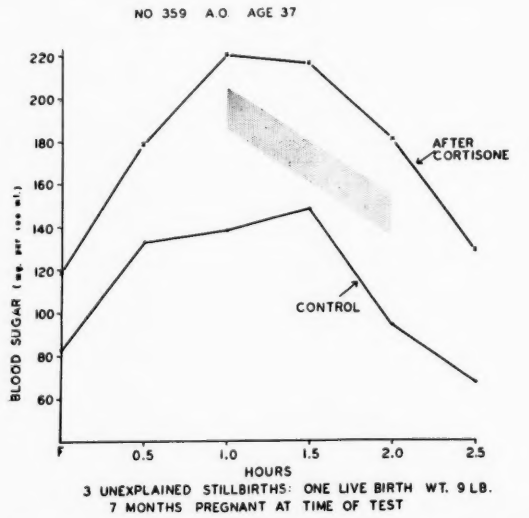


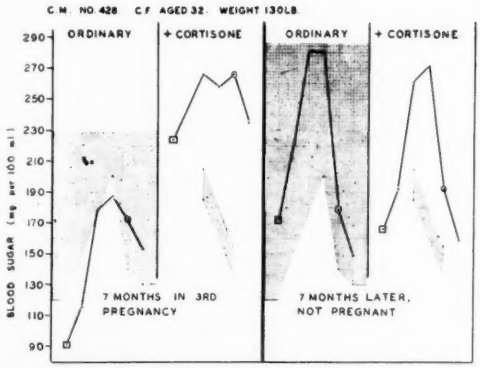
FIG. 6. Note completely normal control curve, but grossly abnormal cortisone curve, in a suspected prediabetic.

diabetic state appeared with each pregnancy. Curve No. 5 of figure 8 illustrates the low normal curve obtained when M.L. was not pregnant, augmented into grossly diabetic realms by cortisone (curve No. 6 of figure 8). This figure, incidentally, shows the remarkable diabetogenic effect of late pregnancy in this girl.

In contrast to the last case is R.H. (No. 317), a rather obese lady, aged twenty-eight. Since both her parents were diabetic, and her first baby had been stillborn, her prediabetic status seemed assured. Her second baby lived, labor having been induced at thirty-five weeks. Serial glucose tolerance curves during a third pregnancy were not grossly abnormal, though one or two were slightly suspicious, with two-hour levels around 140 mg. Cortisone-glucose tolerance tests were performed in 1955 and again at six months during a fourth pregnancy two years later, but even these were not abnormal.

Subjects believed to be prediabetic on basis of family history

Twin brother diabetic. One man, aged thirty-seven, had an identical twin who developed severe insulin-requiring diabetes four years ago. His ordinary and augmented tolerance curves were quite normal.



3 CHILDREN, 6, 9 AND 8 LB. AT BIRTH. MOTHER DIABETIC. GLYCOSURIA IN 3RD PREGNANCY. i-v TOLERANCE DURING PREGNANCY. MEAN INCREMENT INDEX = 2.5 mg. per min.
□ = FASTING
○ = 2 HRS. AFTER 50 gm. GLUCOSE

FIG. 7. The shaded areas in the first and third curves represent the abnormal zone in our interpretation of the ordinary glucose tolerance curve. Note suspicious abnormality in ordinary curve during pregnancy and grossly abnormal cortisone curve. Confirmation of suspicions by grossly diabetic status later, when not pregnant.

Mother, maternal uncles and son diabetic. A man with this family history proved to be a latent diabetic by ordinary glucose tolerance curve. The addition of cortisone did not raise the curve.

Both parents diabetic. 1. R.H. (No. 317, already mentioned). Her presumed prediabetic state could not be brought out by pregnancy or cortisone.

2. A.K., aged twenty-eight (figure 9) had given birth to one normal seven-pound infant. She was first tested when eight months pregnant in her second pregnancy. Ordinary glucose tolerance was normal, but the augmented curve plainly abnormal. On repeating the tests when *not* pregnant, Mrs. K yielded completely normal curves. In view of our findings in the pregnancies in some normal control women, the interpretation of Mrs. K's results is doubtful.

3. Two sisters, aged twenty-four and thirty (Nos. 386, 387) had completely normal ordinary and augmented tests. They were not pregnant when tested.

4. In a further sibship of eight members aged thirty-seven to fifty-four, two were known diabetics, four were latent diabetics with grossly abnormal ordinary glucose tolerance curves, and in one the curve was plainly abnormal after cortisone. The final sib was

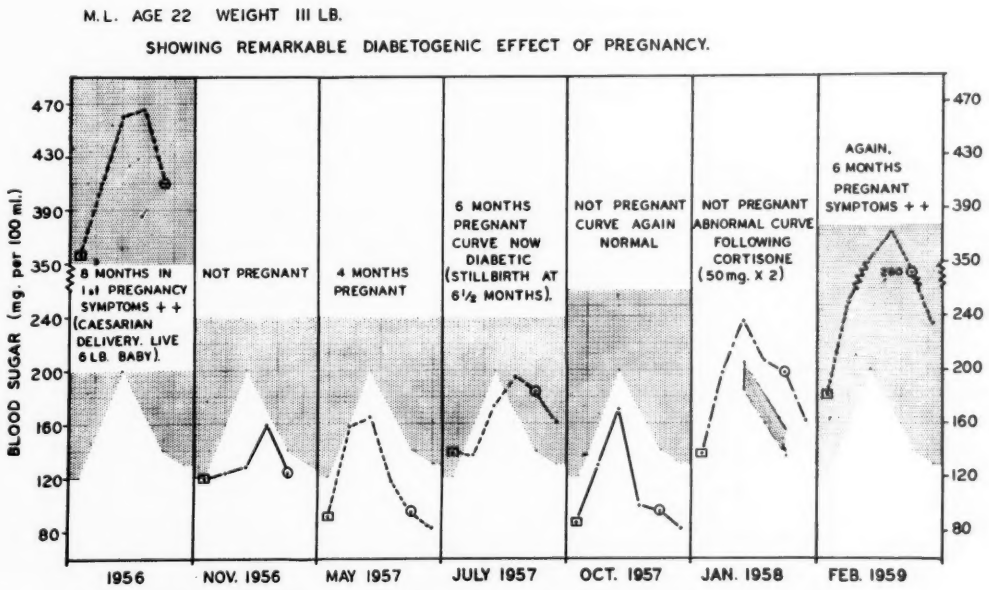


FIGURE 8

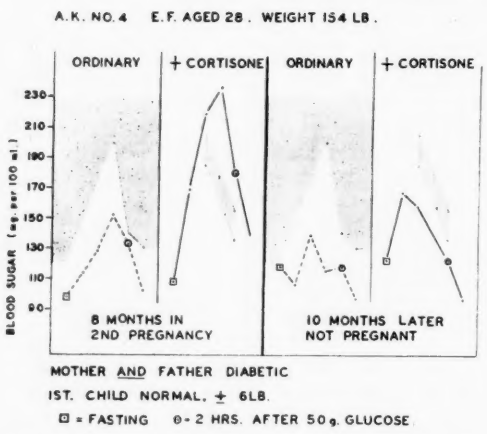


FIG. 9. Note that cortisone curve is abnormal during pregnancy, but normal afterwards.

a woman of forty-one, whose control and augmented curves were normal, yet her three babies had each weighed over ten pounds at birth. In this family, then, all the eight sibs were apparently either diabetic or potentially diabetic. The cortisone test confirmed the diagnosis in one case, but yielded a false negative response in another.

Mild diabetics (thirty-eight subjects)

The mean curves of the first seven subjects in this group are illustrated in figure 10. Cortisone produced a mean increase of about 60 mg. per 100 ml. at one hour. However, some mild diabetics showed no raising of the curve after cortisone. To date the tolerance curves of thirteen patients out of thirty-eight in this group

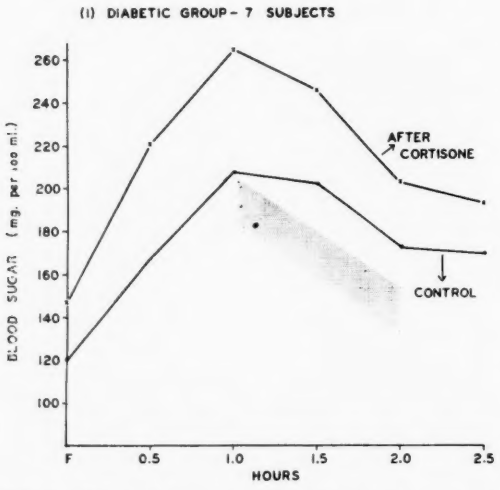


FIG. 10. The usual response of the mild diabetic, as shown by mean curves in the first seven subjects tested.

have been unchanged by cortisone, and the mean curves of the first six of these subjects are shown in figure 11.

In three patients whose diabetes was in remission after loss of weight, plainly diabetic curves were produced by the cortisone test.

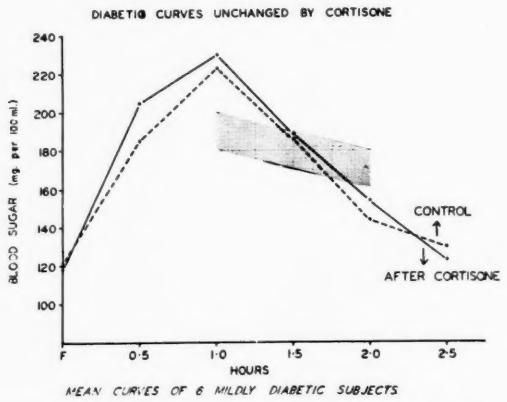


FIG. 11. The shaded area should be disregarded here. Note there is no effect of cortisone on glucose tolerance in some mild diabetics.

DISCUSSION

Effect of corticoids on glucose tolerance

A priori it is unlikely that a single or double dose of cortisone should be sufficient to produce a definite diabetic curve in all prediabetics. This can be seen by consideration of the development of steroid diabetes during therapy. We believe that those who do become diabetic while taking glucocorticoids in moderate doses were previously prediabetic subjects.[8] Certainly most patients who develop diabetes during therapy lose it when the treatment is stopped, but they may again become diabetic later. Some patients become diabetic on steroids within two days, others not for many months. It is thus unlikely that a single test will always be sufficient to bring out an innate diabetic tendency.

The likely value of the test is lessened by the finding of the large proportion of definite diabetics whose curves are not worsened by cortisone. In fact the mild diabetics we have tested appear to be rather distinctly divided into two classes—those whose glucose tolerance is unaltered and those whose tolerance is grossly worsened by cortisone. The interpretation of this is not clear.

Effect of age: Are different standards warranted for older people?

There seems to be no doubt that a group of people aged forty-five years or older, selected only in that they were not known or suspected diabetics (without a

family history of diabetes), possess a mean glucose tolerance which is considerably less than a younger group, and also show a greater response to cortisone-priming. Is this simply because of the inclusion of a number of true diabetics whose disease at this age has become manifest, and could these subjects then be excluded from the statistics? I do not believe this would be valid, because the small group of curves classed as "diabetic" were not sharply cut off from the rest; intermediate between them and the normal range lay another group of curves, which were not normal but yet not definitely diabetic. The same argument holds with the cortisone curves; here we should have to exclude over one third of the total.

We are drawn, then, to the conclusion that the pancreatic β cell reserve may, in general, be reduced over the age of forty-five, and this reduction is apparently accentuated by cortisone priming. Alternatively, the peripheral utilization of glucose may be diminished, by loss of tissue sensitivity to insulin, rather than by deficiency of this hormone. Statistically we must then say that a definitely abnormal response to the augmented test in this age group should lie above 230 mg. at one hour and 215 mg. at two hours (our mean figure plus twice the S.D.).*

Effect of pregnancy

The so-called diabetogenic effect of pregnancy is well known. This effect may, in part at least, be connected with the high levels of circulating glucocorticoids which obtain in the latter half of pregnancy, although it is doubtful whether all the steroid measured is physiologically active. It is interesting, then, to find that our standard dose of cortisone raises the glucose tolerance curve more in the pregnant woman than in the nonpregnant, whereas the naturally occurring glucocorticoids during pregnancy make virtually no difference—in late pregnancy, when the corticoids are at their highest levels, we have actually found the mean tolerance curve to be slightly lower than in the normal nonpregnant state.[7] It is also interesting that there is some evidence that, using other criteria (e.g., output of 17-ketosteroids, electrolyte changes and eosinophil levels), women during pregnancy appear *less* sensitive to cortisone or ACTH.[10] Pregnant rats have been claimed to be extremely cortisone-resistant.[11]

The susceptibility of pregnant women to the cortisone effect may be used to advantage by performing the test during a pregnancy in a suspected case of prediabetes. Thus, the four patients whose prediabetes was established on obstetrical grounds, and in whom the augmented test was grossly abnormal, were pregnant at the time of this test (e.g., figure 6). Unfortunately, however, the frequent raising of the tolerance curve by cortisone in those pregnant women in the control group who were unlikely to be prediabetic (i.e., probable false positive responses), must make one wary of accepting the results of the augmented test as final. It is, of course, just possible that these latter women were really prediabetic and that only the combination of pregnancy and cortisone would indicate this condition. Only long follow-up can decide this question.

Value of test in predicting diabetes

In subjects whom we believe to be almost certainly prediabetic, we have found the test to be of only occasional value in confirming the diagnosis. It seems certain that a negative result does not rule out the possibility. Is a positive result (gross raising of the curve) necessarily indicative of future diabetes? This can only be answered by time, but false positive responses seem to be quite likely in older people or during pregnancy. In older people, the test may still be of value provided the criteria of abnormality are raised.

Conn[12] reported in 1958 that thus far four out of thirty of his abnormal reactors to the augmented test who had been followed up had become grossly diabetic. He made the interesting suggestion that the cortisone-glucose tolerance test might be employed routinely for the diagnosis of diabetes in place of the ordinary test, with the basic idea that a so-called "prediabetic" needed consideration and treatment similar to the established diabetic. The apparent existence of false positive tests unfortunately militates against this suggestion.

SUMMARY

Some of our results with the cortisone-augmented glucose tolerance test are presented. It appears that many false negative results may be obtained, since a number of definite mild diabetics and others who are almost certainly prediabetic show no worsening of glucose tolerance after cortisone. Occasionally the test may be useful in confirming prediabetes, suspected on obstetric grounds, especially if it is performed during pregnancy. Normal pregnant women and older people, however, appear to be more sensitive to the effects of cortisone. During pregnancy, the ordinary glucose tolerance test

*If pregnancy is truly diabetogenic, it is possible that the inclusion of many highly parous women in this control group would raise the mean tolerance curves. The effect of parity on glucose tolerance is being investigated at present.

is not worsened, but the added stress of cortisone seems to overcome pancreatic reserve in some apparently normal women. In the group forty-five years of age and over, on the other hand, the mean ordinary tolerance curve is considerably higher than in the younger group, and the raising by cortisone is also greater. It is suggested that different standards of abnormality must be used if this test is applied to older people. The augmented test is difficult of interpretation and cannot be recommended for routine use.

ACKNOWLEDGMENT

I am grateful to all colleagues who have referred patients to me, and especially to Dr. J. Herman; to the subjects who submitted themselves to the tests, and to Miss M. Lloyd and Mr. I. O'Reilly, who carried out most of them. Most of the expenses were defrayed by grants from the South African Council for Scientific and Industrial Research and the Wellcome Trust. I also acknowledge the assistance of Mr. B. Todt, Mrs. A. Robertson and Mrs. E. Orkin in the preparation of the figures and the manuscript.

SUMMARIO IN INTERLINGUA

Le Test de Tolerantia pro Glucosa Augmentate de Cortisona con Referentias Special al Prediction de Diabete: Diagnose de Prediabete

Es presentate certes de nostre resultatos obtenite in le test de tolerantia pro glucosa a augmento de cortisona. Il pare que numerose reactiones falsemente negative pote esser obtenite, proque un numero de subjectos definitemente con leve diabete e alteres qui es quasi securmente prediabetic non manifesta un pejoration de lor tolerantia pro glucosa post administrationes de cortisona. In certe situationes le test es forsan utile como productor de evidentia confirmatori de prediabete que ha essite suspicite super le base de constatationes obstetric, particularmente si le test es effectuate durante le pregnantia. Tamen, gravidas normal e subjectos de etate plus avantiate es apparentemente plus sensibile al effecto de cortisona. Durante le pregnantia, le ordinari test de tolerantia pro glucosa non es pejorate, sed le stress additional del cortisona pare capace a exhaurir le reservas pancreatic in certe feminas de apparentia normal. In le gruppo de etate de quaranta-cinque annos e plus, del altere latere, le curva del tolerantia medie pro glucosa sin cortisona es plus alte que in le gruppo plus juvene, e le augmento causate per cortisona es etiam plus marcate. Es proponite que differente standards de anormalitate debe esser usate si iste test es applicate a subjectos de etate plus avantiate. Le augmentate test es difficile a interpretar e non pote esser recommendate pro uso routinari.

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The Effect of Prednisolone on Glucose Tolerance in Respect to Age and Family History of Diabetes Mellitus

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The familial tendency of diabetes mellitus has long been known. Many investigators[1,2,6,11,12,15,16,18] have suggested that the predisposition to this disorder of metabolism is inherited as a Mendelian recessive character. Neel[10] pointed out that, as well as an increased incidence of diabetes mellitus among the relatives of diabetic patients when compared with nondiabetics, there are also large numbers of abnormal glucose tolerance curves indicative of impaired carbohydrate metabolism in apparently normal relatives of diabetics. His main interest was in the possibility of detecting genetic carriers and he suggested that potentially diabetic patients might transmit the tendency to their descendants. Iannaccone and others[7] studied hereditary factors and age of onset of diabetes and their findings appeared to confirm the hypothesis of recessive inheritance of diabetes mellitus. This theory was supported by Steinberg,[14] who also pointed out that a high percentage of individuals genetically liable to diabetes are not recognized and that there is no method of predicting when such persons will become clinically diabetic. In 1955 Jackson[8] suggested that an obscure disorder of carbohydrate metabolism is present from birth and, at a later date, this may be clinically recognized as diabetes mellitus. Conn and Fajans[5] have made a significant contribution by comparing glucose tolerance before and after loading doses of cortisone in two groups, one showing some family history of diabetes and the other none. They found that a significant number of nondiabetic relatives of diabetic patients showed decreased glucose tolerance following cortisone, when compared with the control series of subjects with no diabetic family history. Further observation of those originally tested has suggested that diabetes occurs more frequently in the positive reactors than in those who have no decrease of carbohydrate tolerance after loading with cortisone.[9] West[17] performed a similar study and confirmed Conn's finding that a significant number of nondiabetic subjects with a family history of diabetes showed a decreased carbohydrate tolerance after loading with cortisone, when compared with a group of normal controls. He also pointed out that a "positive" response to this steroid-glucose test was found more frequently in older than in younger subjects. It is well to remember that in 1924 McLean[9] pointed out that in apparently normal individuals there was a gradual decrease in carbohydrate tolerance with increasing age. The present investigation is concerned with a modification of Conn's technic to study the effects of prednisolone on glucose tolerance in nondiabetic relatives of diabetic patients in various age groups.

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SUBJECTS AND METHODS

In all, seventy-seven persons were investigated. All were ambulant and free of any symptoms or signs suggestive of metabolic disorder. They were divided into two groups, (a) one with a family history of diabetes mellitus and (b) the other with no known family history of the disease. Each of these groups was then subdivided into three groups according to age (table 1).

Group 1 (under twenty years). Fourteen controls and sixteen relatives of diabetics were studied. The mean age of the controls was seventeen years with a range of nine to nineteen years and of the diabetic relatives sixteen years with a range of eleven to nineteen years.

Group 2 (twenty to forty years). Here the twelve controls had a mean age of twenty-six years with a range from twenty-one to thirty-eight years and the eighteen relatives of diabetics a mean age of twenty-seven years with a range from twenty to thirty-nine years.

Group 3 (over forty years). In this group the mean age for the nine controls was sixty-two years with a range from fifty-one to seventy-five years and for the eight relatives of diabetics forty-eight years with a range from forty-two to fifty-three years.

The study was made under normal conditions of en-

TABLE 1

	Total		Group 1 under 20 years		Group 2 20 to 40 years		Group 3 over 40 years	
	Controls	Diabetic relatives	Controls	Diabetic relatives	Controls	Diabetic relatives	Controls	Diabetic relatives
Number of patients	35	42	14	16	12	18	9	8
Mean age (years)	32	29	17	16	26	27	62	48
Age range (years)	9 - 75	11 - 53	9 - 19	11 - 19	21 - 38	20 - 39	51 - 75	42 - 53
Sex distribution								
Male	12	20	1	8	10	9	1	3
Female	23	22	13	8	2	9	8	5

vironment and, although no rigid dietary control was exercised, each subject was closely questioned to ensure that he or she had taken a reasonably well balanced diet with adequate carbohydrate. Two glucose tolerance tests were performed on each subject under these conditions. All were fasted for twelve hours and a sample of capillary blood was then taken. They were immediately given 1 gm. of glucose per kg. body weight in a 20 per cent aqueous solution by mouth and samples of capillary blood were taken half-hourly for the following two hours. The interval between the standard tolerance test and the prednisolone tolerance test in every instance was between three and five days. Before the second test all were given 1 mg. of prednisolone per fourteen pounds body weight twelve hours and again two hours before the test commenced. The blood glucose estimations were made by the method of Somogyi.[13]

RESULTS

The results were examined with regard to changes at each individual point of the glucose tolerance test (GTT). From this preliminary study it was decided that the only changes worthy of consideration were those observed during the second hour. A difference of more than 20 mg. per 100 ml. between the pre- and post-prednisolone blood glucose values at any two of the three points one, one and one half and two hours was arbitrarily taken to indicate a positive result. Figure 1 shows the pre- and post-prednisolone curves of negative reactors among diabetic relatives and these are seen to coincide over these points.

The results from the complete series are shown in figure 2. It is seen that there is an elevation in the GTT following the administration of prednisolone in both controls and relatives of diabetics, but that this is more marked in the relatives when the second half of the curve is examined.

However, when these results are considered in the three age groups, it is seen that most of the significant

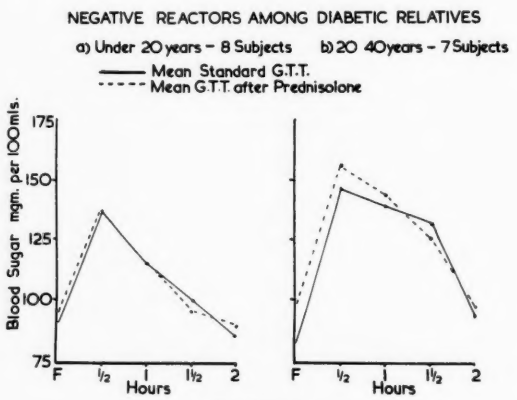

FIGURE 1

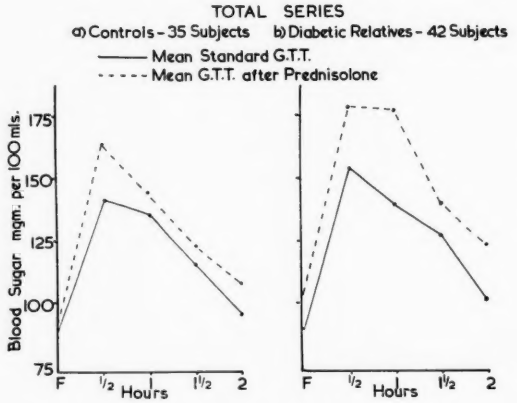

FIGURE 2

decrease in the tolerance of controls occurs because of the large number of positive reactors in the group of control patients over the age of forty years.

The results for Group 1 (under twenty years) are shown in figure 3, where there is a slight elevation in the second half of the mean prednisolone GTT in the

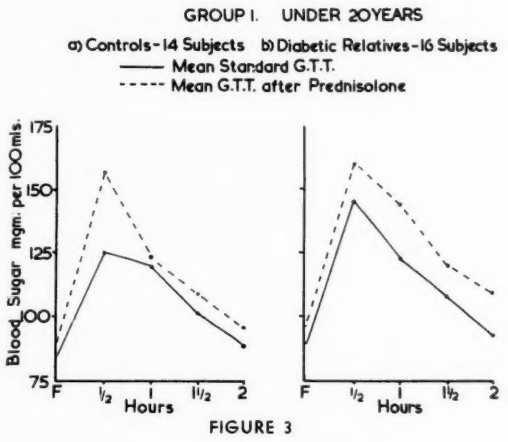

FIGURE 3

controls, among whom there was one positive reactor. By contrast there is conspicuous elevation of the mean prednisolone GTT in diabetic relatives, of whom eight of sixteen were positive reactors.

Figure 4 shows the results for Group 2 (twenty to forty years). There is an insignificant elevation of the mean GTT after prednisolone in the controls, only one of whom was a positive reactor. By contrast, the mean GTT following prednisolone in the relatives of diabetics is markedly elevated because eleven of eighteen were positive reactors.

In Group 3 (over forty years) the controls and diabetic relatives may not be truly comparable because of age differences. However, a positive reaction was given by five of nine controls compared with six of eight diabetic relatives. Consideration of the second half of

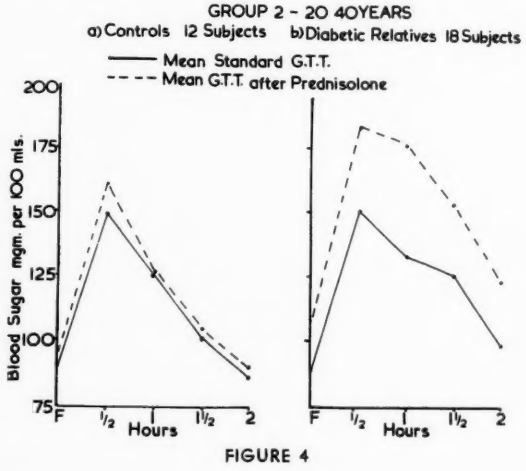

FIGURE 4

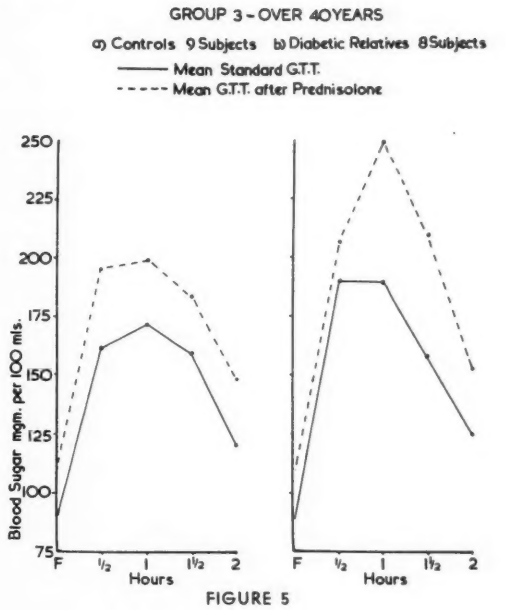

FIGURE 5

the glucose tolerance curves in this group (figure 5) shows that, although the prednisolone GTT in the controls is definitely elevated, the response in the diabetic relatives is much greater.

Table 2 shows positive reactors in diabetic relatives and controls when considered according to age and sex.

DISCUSSION

In any study where age is an unknown factor, difficulties arise in obtaining comparable results. One problem is that of obtaining suitable controls. The group investigated was one of hospital outpatients with some members of staff and some medical students. No healthy children were available for study in a public hospital. For this reason the juvenile controls consisted substantially of a group of female laboratory technicians who were at work during the tests. Another problem in the juvenile group was that a parent of a young child can develop diabetes at a later stage and hence a positive response may be obtained in a subject initially regarded as a control. However, in this series only one of the fourteen juvenile controls has shown a positive response so far.

Healthy adults in the forty to sixty age group were not readily available. To overcome this an attempt was made to use long-term orthopedic inpatients as control subjects at a period not less than three weeks after injury or operation. They showed a marked and uniform

TABLE 2

Positive reactors in relation to age and family history

	Total		Group 1 under 20 years		Group 2 20 to 40 years		Group 3 over 40 years	
	Controls	Diabetic relatives	Controls	Diabetic relatives	Controls	Diabetic relatives	Controls	Diabetic relatives
Number of patients	35	42	14	16	12	18	9	8
Positive reactors								
Number	7	25	1	8	1	11	5	6
Percentage	20	58	7	50	8	61	46	75

loss of carbohydrate tolerance and gave strongly positive reactions after prednisolone, suggesting that either injury or hospitalization may alter tolerance appreciably. The curves in many instances resembled the type observed during the feeding of a high fat diet. Such patients were considered unsuitable.

The controls in Group 3 consisted of hospital outpatients with trivial illnesses having no effect on carbohydrate metabolism. They were somewhat older than the diabetic relatives investigated. Studies in this age group were complicated because of the progressive loss of carbohydrate tolerance with increasing age (figure 6a) which is accentuated by prednisolone (figure 6b).

The results of this investigation confirm the findings of Conn and Fajans[5] in that the relatives of diabetics as a group show a greater decrease in carbohydrate tolerance following the administration of a glucogenic steroid when compared with a control group (figure 2). This decrease is mainly shown during the second hour of the test, a fact also noted by Conn.[4]

The decrease in glucose tolerance in the second hour of the test following prednisolone is seen in all groups of diabetic relatives. The change is not so marked in the juvenile group as in Group 2 (figures 2 and 3). This supports the finding of West[17] who considered the response to this test increased with age. The older group (over forty years) show an even larger response to prednisolone but also show some loss of standard glucose tolerance (figure 5b).

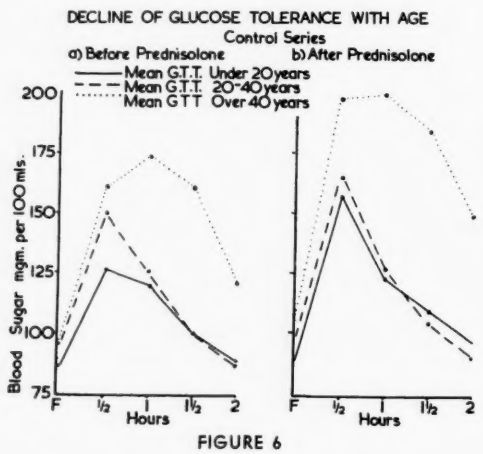

FIGURE 6

Similarly, the older control group show a greater response to prednisolone and a greater number of positive reactors (46 per cent) than any of the other control groups. Since no preselection according to preliminary GTT was employed in this investigation, this group is not comparable to the results reported by Conn and Fajans.[5]

Thus, the test under these conditions can be considered reliable up to the age of forty years, although beyond this age it becomes less reliable. This is due in part to the marked loss of glucose tolerance with advancing age (figure 6). Also, the relatives of diabetics in this age group are often siblings of those who have already developed diabetes and who may well represent the genetic type not liable to the disease.

It is of interest that, in two sets of identical twins studied, both nondiabetic twins fit these criteria in showing a tendency towards diabetes. The first were twin boys aged fourteen years with a direct paternal family history of diabetes. When one twin developed diabetes, a prednisolone GTT was performed on the other. He showed a marked positive response and less than twelve months later developed frank diabetes requiring insulin.

The second pair of twins were girls with no family history of diabetes. One presented with diabetes mellitus in July 1952. At this time, and in 1955, standard GTT's performed on her twin sister were quite normal but in 1957 she showed a positive reaction to a prednisolone GTT. However, she has not shown any sign of diabetes as yet. It is of interest to note the absence of any other family history in her case and its presence in the previous one.

Altogether three subjects studied have developed diabetes since the tests were done. All three were positive reactors under the age of forty years.

It has been observed that in both the control and experimental series the administration of prednisolone resulted in a significant increase in blood glucose of similar magnitude at thirty minutes. No explanation can be offered for this finding. It is possibly due to a more rapid absorption of glucose produced by the action of the steroid.

Consideration of the results obtained emphasizes the necessity of proper selection of controls and the fallacy of the use of a control group within narrow age limits (such as students) for this type of study. Also, MacLean's[9] finding of decreasing glucose tolerance with increasing age is too often forgotten.

Only a prolonged follow-up can show whether those diabetic relatives giving a negative response to the test are less prone to develop the disease than their fellows with a positive response, and thus give a final evaluation of the test.

SUMMARY

The effect of prednisolone on glucose tolerance has been studied with respect to age in forty-two subjects with a diabetic family history and compared with a control group of thirty-five subjects with no diabetic family history.

It has been found that prednisolone produces a greater impairment of glucose tolerance in the relatives of diabetics than in the control series. The significant changes in the prednisolone GTT are found in the second hour. The test appears reliable up to the age of forty years. The possible significance of the findings is discussed.

SUMMARIO IN INTERLINGUA

Le Effecto de Prednisolona Super le Tolerantia pro Glucosa, con Respecto al Etate e al Historia Familial de Diabete Mellite

Le effecto de prednisolona super le tolerantia pro glucosa esseva studiate con respecto al etate in quarantaduo subjectos con historias familial de diabete e comparate con un gruppo de controlo de trenta-cinque subjectos sin historia familial de diabete.

Esseva trovate que prednisolona produce un plus marcate disturbation del tolerantia pro glucosa in consanguineos de diabeticos que in le subjectos de controlo. Le alterationes significative in le test de tolerantia pro glucosa a prednisolona se incontra in le secunde hora. Le test pare esser digne de confidentia usque al etate de quaranta annos. Le signification possibile del constatationes es discutite.

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Motor Nerve Conduction Velocity in Diabetes Mellitus

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Disturbances in neurological function are among the most common of the complications of diabetes. The reported incidence of neurologic involvement varies from 25 to 90 per cent of adult diabetic patients.[1-6] The severity of the symptoms associated with diabetic neuropathy may vary from a degree so mild as to be unnoticed to a disorder so distressing that chronic invalidism results. The most common neurologic disturbance in diabetes is involvement of the peripheral nerves.

The early symptoms of peripheral nerve involvement are usually sensory. Evaluation is often difficult because of variation in the patients' interpretation of the symptoms, as well as the inherent subjectivity of sensory examination.

Therefore, a physical method for documentation and quantitation of peripheral nerve function could be of great aid in the study of diabetic neuropathy. The precise measurement of the conduction velocity of peripheral motor nerve fibers offers such a method. Even though many of the clinical disturbances in diabetic neuropathy are sensory, it could be postulated that altered excitability of the efferent fibers may occur without obvious weakness of muscles supplied by these motor fibers and that conduction velocity may reflect the general state of function of the peripheral nerve. Our studies utilizing measurement of nerve conduction velocity in 103 diabetic patients appear to confirm this hypothesis.

METHODS

Determinations of the conduction velocity of the peripheral motor fibers in the ulnar and peroneal nerves were made in 103 diabetic patients selected from the outpatient clinic or from hospitalized patients admitted to the division of Endocrinology and Metabolism of Ohio State University Hospital. Selection of diabetic patients was dependent chiefly on the individual patient's willingness and ability to take part in the study. Occasionally patients were included because they presented neurological findings which were felt to be of special interest.

Prior to the measurement of nerve conduction velocity, the clinic record of each patient was reviewed. Following this, a supplemental history was taken and a physical examination which included a special neurological examination was performed.

On the basis of these findings, an evaluation of the various degenerative complications of diabetes was made. Where necessary, these evaluations were substantiated by laboratory investigation. Subjects with either retinal microaneurysms, waxy exudates, or both were considered to have "diabetic retinopathy." Individuals having sustained diastolic blood pressure levels exceeding 90 mm. Hg in the supine position were classed as "hypertensive." Those who related a clear-cut history of angina pectoris or displayed electrocardiographic evidence of myocardial infarction were considered to have "arteriosclerotic heart disease." A diagnosis of "peripheral vascular disease" was made in subjects who presented a clear-cut history of intermittent claudication or showed definite signs of arterial insufficiency, such as, absent foot pulses or dependent rubor. The findings of edema unexplainable on the basis of congestive heart failure, proteinuria in excess of 20 mg. per cent, and elevation of the BUN above 20 mg. per cent were taken as presumptive evidence of diabetic "nephropathy."

For purposes of standardization, the neurological evaluations of the patients were made by the same individual. Physical findings such as the biceps, triceps, patellar and Achilles tendon reflexes and perception of light touch, painful stimuli and vibratory stimuli, were noted as either present or absent. Functional tests to evaluate motor function of the lower extremities were

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performed in all patients. They included walking on heels and toes, squatting and rising. Patients having any of these neurologic defects were considered to have "objective neuropathy." Symptoms such as numbness, paresthesias, hyperesthesias, and spontaneous pain sensations were recorded. Patients having symptoms only were grouped as "subjective neuropathy." Patients who showed decrements in blood pressure which exceeded 10 mm. Hg when rising from a supine position were classified within a "visceral neuropathy" group. All patients who met the criteria for inclusion in any of these groups were considered to have evidence for the diagnosis of clinical neuropathy. The findings and characteristics of these patients were then compared to those of the diabetic subjects who were not considered to have clinical neuropathy and to a group of nondiabetic patients.

Peroneal nerve conduction velocity was determined in one leg and in the ulnar nerve in the opposite arm. In selected individuals, repeated determinations were made. Normal values were established by measuring ulnar and peroneal nerve conduction velocity in a group of volunteers known to be free of neurological disease and of approximately the same age group as that of the diabetic patients.

Electromyographic studies, employing conventional technics, were done in a majority of patients selected at random.[7]

Percutaneous electrical stimulation of the peroneal and ulnar nerves was carried out in the following manner: The peroneal nerve was stimulated with bipolar electrodes at the knee and the ankle. The muscle action potential was picked up with 5 mm. cup-shaped surface electrodes on the skin over the extensor digitorum brevis. The reference electrode of similar construction was placed over the tendon of this muscle. A photograph of the oscilloscope screen showing the stimulation artifact and the amplified muscle action potential with an appropriate time base was taken. This was repeated three times at each stimulation point to rule out variation by patient movement. The distance between the artifact and the action potential was determined by inspection of the photograph. The distance on the skin between the points of stimulation was measured, and the conduction velocity was calculated in meters per second.

A similar procedure was carried out with the ulnar nerve, except that the stimulation was done at the elbow and at the wrist, and the pick-up electrode placed over the abductor digiti quinti.

The apparatus (figure 1) comprised a standard laboratory stimulator, a pre-amplifier, an oscilloscope with a polaroid camera, an audio amplifier and a loudspeaker, a

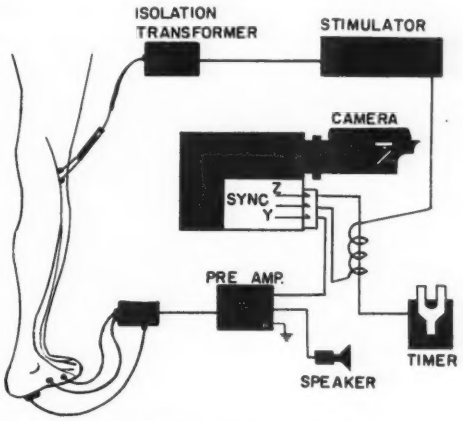

FIG. 1. Schematic diagram of apparatus used to measure nerve conduction velocity.

sturdily constructed set of stimulating electrodes, and electroencephalographic surface electrodes. The timing device was a tuning fork with a power supply, whose voltage applied to the "Z" axis of the oscilloscope, blanked out the sweep at one-millisecond intervals to provide the time base. Intramuscular temperature in the vicinity of the nerve was taken with a thermistor mounted in a twenty-five gauge hypodermic needle. Since reduced temperatures result in diminished conduction velocity, all results were corrected for temperature reduction (5 per cent/degree C.).

The procedure required only several minutes in a cooperative patient. Occasionally technical difficulties arose when the patient had edema of the foot and ankle. A description of this technic has been previously reported.[8]

RESULTS

A. Group Characteristics and Incidence of Neurologic Findings. The fifty-six women and forty-seven men studied had an average age of 51.9 years and an average duration of diabetes of 7.9 years (table 1). Two thirds had one or more degenerative complications of diabetes. The frequency of complications is similar to that reported in other series of diabetic studies.[2,3,5,9,10] As a group, the patients studied had a somewhat larger than usual insulin requirement; 51 per cent took insulin in an average daily dose of 44 U.

The frequency of the various symptoms and signs believed to be associated with diabetic neuropathy is depicted in figure 2. The incidence and variety of the neurological findings in the entire group and in patients with "diabetic neuropathy" are in general agreement with those reported in the literature.[1-3,5,9,10]

TABLE 1

Characteristics of the 103 diabetics studied

Age: Average—51.9 years, range—12 to 87 years
Duration of diabetes: Average—7.9 years, range—1 to 35 years
Sex: Female—54 per cent, Male—46 per cent

Associated complications	Per cent
Nephropathy	12
Retinopathy	23
Arteriosclerotic heart disease	28
Peripheral arteriosclerosis	28
Hypertensive cardiovascular disease	40
Therapy	
Diet only	7
Oral agents	
Chlorpropamide	29
Tolbutamide	7
Phenformin	4
Insulin (average dose 44 U.)	52

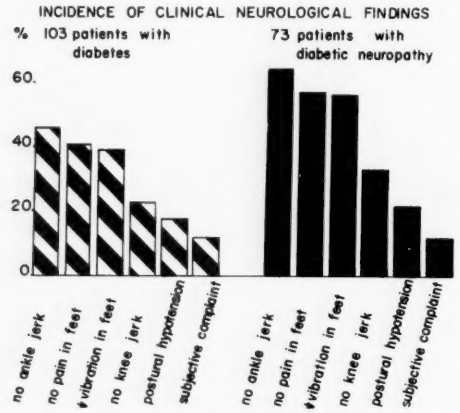

FIGURE 2

B. Nerve Conduction Velocity in Diabetics and Normals. Mean normal values for ulnar and peroneal nerve conduction velocity were 56.4 ± 6.2 and 49.3 ± 5.7 m./sec., respectively. These values compare favorably with those reported in the literature.[7,8] In diabetic patients the mean values for both ulnar and peroneal nerve conduction velocity were significantly below normal. Ulnar nerve conduction velocities averaged 48.6 m./sec. or 14 per cent less than that of the normal group. Peroneal velocity averaged 38.6 m./sec. or 22 per cent less than normal. When comparison was made between the group of diabetics with clinical neuropathy and non-diabetic normals, these differences became even greater. Figure 3 shows the distribution of ulnar nerve conduction velocities of diabetic patients with neuropathy and those of normals. The normal patients averaged 56.4 m./sec. and in 87 per cent of them the value exceeded 50 m./sec. In the diabetic neuropathy group, 77 per cent had a nerve

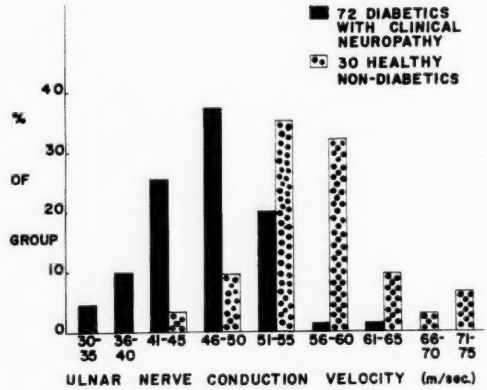

FIG. 3. Comparison of ulnar nerve conduction velocity in diabetic patients with neuropathy and normal patients.

conduction velocity of less than 50 m./sec.

An even greater difference between patients with neuropathy and normals was found when values of peroneal nerve conduction were studied. The seventy-three patients with neuropathy were found to have a mean of 36.5 m./sec. compared to 49.3 m./sec. Only 3 per cent of the normal group had values below 40 m./sec., but 77 per cent of the diabetic neuropathy group were below this level (figure 4).

When comparison of diabetics without clinical neuropathy and the normal group was made, values for both ulnar and peroneal nerve conduction were again lower (53.5 and 43.6 m./sec. respectively).

Statistical analysis of these data indicate significant differences for both ulnar and peroneal values in diabetic neuropathy patients compared to normal. In the group of diabetics without neuropathy, peroneal but not ulnar nerve conduction velocity was statistically different. These findings are listed in table 2.

C. Relationship of Nerve Conduction Velocity to Clinical Status. There was general agreement between the clinical findings interpreted as peripheral neuropathy and reduction of motor nerve conduction velocity. When values of two standard deviations below the mean of the normal group were regarded as reduced, peroneal conduction velocity was found to be reduced in 77 per cent of patients considered to have neuropathy and was within normal limits in 83 per cent of patients considered to be free of neuropathy (figure 5). In the ulnar nerve reduced values were found in only 35 per cent of patients with neuropathy and were also found in 10 per cent of patients without neuropathy.

The degree of reduction of velocity was found to be

TABLE 2

Statistical analysis of peroneal and ulnar conduction velocity

Nerve studied	Patient group	No. pts.	Mean velocity (m./sec.)	"P" compared to control
Peroneal	Diabetics with neuropathy	73	36.5±5.1	<.01
	Diabetics without neuropathy	30	43.6±6.0	<.01
	Nondiabetic controls	41	49.3±5.7	—
Ulnar	Diabetics with neuropathy	72	46.6±5.9	<.01
	Diabetics without neuropathy	30	53.5±6.2	>.05
	Nondiabetic controls	31	56.4±6.3	—

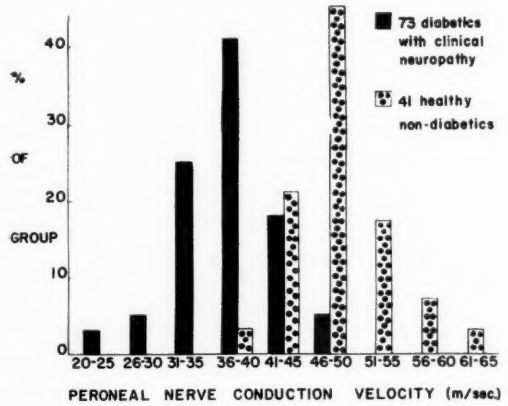

FIG. 4. Comparison of peroneal nerve conduction velocity in diabetic patients with peripheral neuropathy and normal patients.

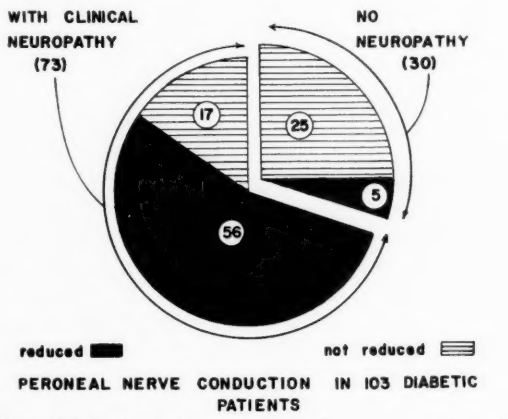

FIG. 5. Relationship between reduction of peroneal velocity and clinical neurological status (see text).

related to the severity of the neuropathy. For example the aggregate of patients having combinations of findings such as absence of patellar reflexes plus loss of pain and vibratory sense in the feet showed a mean peroneal conduction velocity of 31.5 m./sec. compared to one of 40.9 m./sec. found in diabetics free of these neurologic changes.

Most of the patients with no objective neurologic findings but with distressing "subjective neuropathy" (complaints of shooting pains, paresthesias, burning feet, and the like) had peroneal nerve conduction values below normal. These individuals averaged 35.5 m./sec., a value no different from that of patients with "objective neuropathy."

The ages and sex of the patients studied were also related to both the severity and incidence of neuropathy and the nerve conduction velocity. The frequency of clinical neuropathy increased with age. Only 45 per cent of the patients under forty years of age had neuropathy while all those over seventy were affected. Reductions in nerve conduction velocity roughly paralleled this trend. When the sexes were compared men were found to have a more severe clinical neuropathy as well as a greater reduction in both peroneal and ulnar conduction velocities.

D. Electromyographic Findings. Electromyographic studies were completed in seventy-eight patients. In twenty-six the findings were abnormal. Reduction in the number of motor unit action potentials was the most common abnormality. An increased proportion of polyphasic potentials was noted less often, and only in patients with severe neuropathies such as foot-drop were fibrillation potentials observed. All patients with abnormal electromyograms had clinical neuropathy and all but three had reduced peroneal conduction velocities (figure 6).

E. Relationship of Nerve Conduction to Complications Other Than Neuropathy. As noted above, degenerative complications of diabetes were present in exactly two thirds of the patients studied. Even though clinical neuropathy was present in twenty-seven of the thirty-six patients who were free of other complications, their mean peroneal conduction velocity was 40.6 m./sec., a value only 2 m./sec. higher than the average for all diabetics.

When neither neuropathy nor other complications existed, the mean value approached normal (44.1 m./sec.). On the other hand, the mean values for peroneal conduction were distinctly reduced when any of the several complications were present (table 3).

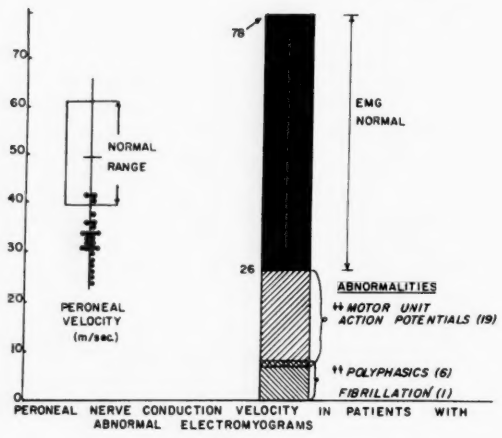

FIGURE 6

TABLE 3
Relationship of degenerative complications to peroneal nerve conduction velocity

Complication	Mean velocity m./sec.	Per cent reduction from normal
None	44.1	10
Neuropathy only	40.6	18
Hypertensive cardiovascular disease	38.5	22
Arteriosclerotic heart disease	37.9	23
Nephropathy	37.1	24
Peripheral arteriosclerosis	36.2	26
Retinopathy	34.9	29

DISCUSSION

The concept of measuring human motor nerve conduction velocity is not a new one. Helmholtz in 1852 measured the velocity of the median nerve and found about the same results as are found with the current apparatus.[8] Refinements in equipment have added precision and facility so that during the past two decades motor nerve stimulation with recording of the muscle action potential has been employed in the investigation of many disorders of the motor unit.[11–15] The application of this technic for the study of the peripheral nerves in patients with diabetes mellitus follows this trend. Mulder and co-workers are also engaged in this phase of investigation.[16]

The reduction of peroneal nerve conduction velocity in patients with neuropathy was anticipated and the degree of reduction in severely affected patients was similar to that observed in chronic peripheral neuropathies due to other causes.[8] The data obtained with respect to peroneal conduction velocity indicate that the degree of reduction is usually proportional to the severity of neurological involvement and that the measurement of motor function frequently reflects the state of neurological function of the lower extremity. The observation that diabetic patients without clinical manifestations of neuropathy may also have reduction of peroneal velocity suggests that in some instances dysfunction of the motor fibers of the peripheral nerve may occur in the absence of either sensory involvement severe enough to provoke symptoms or of motor involvement severe enough to permit the diagnosis of muscle weakness. Since clinical manifestations of neuropathy in the upper extremities were extremely uncommon, but reduction of ulnar velocity was noted often, similar states of subclinical neuropathy probably exist in the peripheral nerves of the arms of many diabetic patients with and without neuropathy of the lower limbs.

The finding that certain patients who exhibit abnormal neurologic signs may have normal conduction velocity values suggests that these individuals have only involvement of sensory fibers. It is also possible that clinical evaluation of symptoms may have been misleading and that some of these subjects may not have had neuropathy. On the other hand the demonstration of reduced conduction in the majority of patients who displayed subjective neuropathy suggests that motor fiber involvement frequently accompanies sensory fiber involvement.

Since abnormalities in the motor nerve conduction velocity were noted in 71 per cent and electromyographic abnormalities in 33 per cent of the patients' studies in our series, the former must be regarded as a more sensitive index of nerve involvement.

The precise relationship of degenerative complications other than neuropathy to nerve conduction velocity is not clear as most patients with one or more complications also had neuropathy. Nevertheless, both the severity of the neuropathy and reduction of velocity were greater in patients with complications. It is probable that peripheral neuritis is more severe in patients with clinical cardiac, renal and peripheral arterial diseases than in those without them.

SUMMARY

Motor nerve conduction measurements were made in 103 diabetic patients who averaged 51.9 years of age and had a mean duration of diabetes of 7.9 years. Seventy-three of these patients were found to have clinical evidence of peripheral neuropathy and a like number had other degenerative complications of diabetes. Values for ulnar nerve conduction velocity in patients with neuropathy averaged 46.6 ± 5.9 meters per second compared to 56.4 ± 6.3 m./sec. for normals. The mean values for peroneal velocity were 36.5 ± 5.1 m./sec. in dia-

betics and 49.3 ± 5.7 in normals. These differences were found to be statistically significant. The aggregate of diabetic patients without clinical evidence of neuropathy was also found to have a reduction in peroneal conduction velocity. The degree of reduction of velocity in clinically involved patients roughly paralleled the severity of the observed neurological deficit, and patients with subjective sensory findings alone had degrees of reduction similar to patients with objective findings. Some patients complaining of subjective sensory disturbances were found to have normal motor nerve conduction velocities indicating that quantitative technics measuring transmission of sensory nerve impulses would also contribute to knowledge of diabetic neuropathy. These findings permit the conclusions that determination of motor nerve conduction velocity provides an objective and quantitative evaluation of peripheral nerve function and, because conduction velocity is significantly reduced in most patients with diabetic neuropathy, it may be used as a guide to diagnosis and management.

SUMMARIO IN INTERLINGUA

Le Velocitate de Conduction del Nervos Motori in Diabete Mellite

Mesurationes del conduction de nervos motori esseva effectuate in 103 diabeticos de un etate medie de 51,9 annos, con un duration medie del diabete de 7,9 annos. Esseva trovate que septanta-tres de iste patientes habeva signos clinic de neuropathia peripheric; un simile numero habeva altere complicationes degenerative de diabete. Le valor medie del velocitate de conduction in le nervo ulnar esseva 46,6 ± 5,9 m/s (metros per secunda), a comparar con 56,4 ± 6,3 m/s in normales. Le valor medie del velocitate peronee esseva 36,5 ± 5,1 m/s in diabeticos e 49,3 ± 5,7 in normales. Esseva constatate que iste differentias es statisticamente significative. Esseva constatate in plus que le aggregato del patientes diabetic sin signos clinic de neuropathia habeva etiam un reducite velocitate de conduction peronee. Le grado de reduction del velocitate in clinicamente afficite patientes esseva grossiermente parallel al severitate del observate deficit neurologic, e patientes con constatationes sensori de typo subjective sol habeva grados de reduction simile a patientes in qui le constatationes esseva objective. Plure patientes qui se plangeva subjectivemente de disturbationes sensori revelava normal velocitates de conduction del nervos motori, lo que indica que technicas mesurante le transmission de impulsos de nervo sensori etiam contribuerea a nostre comprension del neuropathia diabetic. Le datos justifica le conclusion que le determination del velocitate de conduction del nervos motori provide un objective e quantitative evalutation del function del nervos peripheric, e—viste que le velocitate del conduction es significativemente reducite in le majoritate del patientes con neuropathia diabetic—illo pote esser usate como guida in le diagnose e tractamento de iste disordine.

ACKNOWLEDGMENT

Grateful acknowledgment must be given to the following for financial assistance: The National Institute of Arthritis and Metabolic Diseases Training Grant No. 2A-5118 (C2S2); The Comly Coleman Endowment Fund of The Ohio State University; and the Office of Vocational Rehabilitation of the National Institutes of Health, Department of Health, Education, and Welfare.

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Effects of Tolbutamide, Mesoxalate and Phenformin in Vitro on the Liberation of Nitrogen by Rat Liver Slices

Yoshio Goto, M.D., and F. D. W. Lukens, M.D., Philadelphia*

The in vitro effects of tolbutamide on the liver slice and diaphragm of the rat have been extensively studied. The majority of reports record various measurements of the metabolism of glucose. In both isolated tissue and intact animals there is evidence[1,2] that tolbutamide decreases hepatic glucose production, and that this is due in part to a reduced rate of glycogenolysis.[3,8] Since gluconeogenesis from protein is an important component of hepatic glucose production, we have attempted to measure the direct action of tolbutamide on the production of nonprotein nitrogen in vitro by rat liver slices. Similar observations have been made on the rat diaphragm, and the effects of mesoxalate[4,5] and phenformin have been examined by way of comparison.

Kaufmann and Wertheimer[6] have reviewed earlier studies on the liberation of nitrogen by liver slices and have reported the influence of different media and of several physiological conditions on the protein and nonprotein nitrogen output. Their experience will be recalled in connection with our methods and results.

METHODS

Liver slices and hemidiaphragms prepared from 125 gm. male Wistar rats starved eighteen to twenty-four hours were incubated in Krebs-Ringer bicarbonate solution, which contained 200 mg. per cent U-C^{14}-glucose. Kaufmann and Wertheimer[6] found that "liver slices incubated in Krebs-Ringer bicarbonate medium release 48 per cent more total nitrogen than those incubated in Krebs-Ringer phosphate medium," and they describe the effects of other alterations in medium. The presence of 200 mg. per cent of glucose did not change the release of total nitrogen in the phosphate medium which they used. The use of Krebs-Ringer bicarbonate thus appears to be suitable when comparative results are sought, although absolute figures will differ with different media. The effect of tolbutamide or urea was tested by adding sodium tolbutamide in a final concentration of 20 mg. per cent, or urea in a final concentration of 150 mg. per cent. Addition of tolbutamide or urea caused no change in the *p*H of the medium. The rats were killed by cervical fracture. The liver was removed and washed in cold Krebs-Ringer bicarbonate solution which contained no glucose. The left lobe of the liver was sliced with a Stadie-Riggs slicer and the slices were washed in cold Krebs-Ringer bicarbonate solution. The slices were blotted gently on filter paper, weighed, and placed in a Warburg vessel containing 3.0 ml. medium with isotopic glucose. The liver slices weighed about 200 mg. The usual time between sacrifice and incubation was five minutes. Filter papers (20 x 40 mm.) were placed in the center wells of the Warburg vessels which were gassed with a mixture of 95 per cent oxygen: 5 per cent CO_2 for five minutes, and stoppered. The Warburg vessels also contained ground-glass side-arms which were stoppered by rubber self-sealing penicillin stoppers in order that acid and alkali could be introduced into the vessel at the end of the experiment to permit collection of CO_2 as described below. The vessels were shaken for three hours at 37° C. and 80 cycles per minute in a Dubnoff metabolic incubator. After three hours incubation, 0.3 ml. of 9N NaOH was placed in the center well through the side-arm containing the rubber stopper by means of a needle and syringe. Then 0.5 ml. of 4N H_2SO_4 was added directly to the medium with another syringe and the vessels were shaken again at 37° C. for twenty minutes to permit absorption of CO_2 by the filter paper.

Kaufmann and Wertheimer[6] employed Kline's[7] method of deducting the value of an initial incubation period, a procedure designed to account for physical diffusion and leakage due to injury of the tissue. In using the beginning of incubation as the zero value instead of a fifteen-minute[7] or thirty-minute[6] point, allowance has not been made for the large initial output of nitrogen

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due to handling the tissue. This will be discussed in connection with the results.

Determination of the conversion of C^{14}-glucose to $C^{14}O_2$. The filter paper was removed quickly from the center well and placed in a graduated, stoppered centrifuge tube which contained 2 ml. of distilled water. One milliliter of distilled water was used to rinse out the center well and added to the centrifuge tube. The final volume was made to 3.0 ml. A 0.5 ml. aliquot of this was plated, dried and counted.

Determination of incorporation of C^{14}-glucose into glycogen. The liver slice was removed from the vessel, placed in a test tube which contained 1.0 ml. of 30 per cent KOH, and heated in a water bath for one hour. Then two drops of 10 per cent $ZnSO_4$ and 2.5 ml. of ethanol were added, warmed and placed in an ice bath for thirty minutes. The glycogen was precipitated by centrifugation, dissolved in water and reprecipitated by ethanol twice more. The precipitate was then dissolved in 2 ml. of 1N H_2SO_4 and hydrolyzed in a water bath for two hours at 100° C. The hydrolysate was neutralized with 30 per cent KOH and the final volume was made up to 3.0 ml. A half milliliter of this hydrolysate was plated, dried and counted. The other 2.0 ml. was used for glucose determination by the Harding modification of the Schaffer-Hartmann method.

Determination of NPN *in medium.* The medium was transferred to a 15 ml. centrifuge tube by Pasteur pipette and centrifuged. A 2.0 ml. aliquot of the supernatant was added to 2 ml. of 10 per cent sodium tungstate, shaken vigorously, and centrifuged. In the experiments with urea, 2.0 ml. of medium and 2.0 ml. of 10 per cent sodium tungstate were added to a 10-ml. graduated cylinder and the final volume was made to 10.0 ml. with distilled water. After filtration the nitrogen was determined by a micro-Kjeldahl method. Urea was determined by the method of Reinhold and Gentzkow.[8] This measures urea plus ammonia formation by the isolated tissue, but the results are referred to as urea N. Amino nitrogen was determined by the method of Frame, Russell and Wilhelmi.[9] In each experiment with tolbutamide or urea, the initial nitrogen content of the incubating medium was determined in the same way and the appropriate correction made to determine the amount added by the liver slices. In the studies on tissues from pretreated animals, the amounts of drug are noted in the tables. In in vivo studies, tissues were usually taken one to two hours after the last injection of tolbutamide or mesoxalate. Glutamic oxalic transaminase and glutamic pyruvic transaminase were determined in the Pepper Laboratory of the Hospital of the University of Pennsylvania by the method of Karmen[10] and the results reported in Karmen units.

RESULTS

Liberation of nitrogen. Table 1 shows that tolbutamide reduced the nonprotein nitrogen output by liver slices of normal rats. The decrease amounted to 18 per cent. If this were a metabolic effect in the intact animal, it would account for an appreciable decrease in the daily hepatic glucose production from protein. In the diaphragm there was an even greater output of nitrogen, which was less affected by tolbutamide. Sodium mesoxalate exerted no such action on the liver (table 2).

The interpretation of this measurement of nitrogen output requires more knowledge of its metabolic meaning. Kline[7] discussed this and showed that much nitrogen entered the medium during the first fifteen minutes of incubation, and that this initial loss "is not of biological significance." To eliminate this nonmetabolic nitrogen, he used the fifteen-minute period of incubation as the zero point. In addition to the time element, the forms of nitrogen have received consideration. Kline[7] determined total nitrogen. Kaufmann and Wertheimer,[6] using a thirty-minute fore-period, examined the release of protein and of nonprotein nitrogen. Under their experimental conditions (fasting, alloxan diabetes, etc.), the release of nonprotein nitrogen was constant, and protein nitrogen was the variable component. To relate the effects of tolbutamide to these observations,[6,7] total nitrogen and nonprotein nitrogen of the medium were determined at thirty minutes and at three hours and thirty minutes, as shown in table 3. For both the half-hour and three-hour periods the amount of total nitrogen exceeds that of nonprotein nitrogen, confirming the fact that loss of protein is the major source of the nitrogen liberated by these isolated tissues. By estimating the rate per half hour during the final three-hour incubation, one finds that both forms of nitrogen are liberated more than twice as rapidly during the first half-hour, in agreement with others.[6,7] Tolbutamide had no certain effect in the first half-hour and its effect in the subsequent three-hour period was obscured when measured by total nitrogen. However, the definite effect on nonprotein nitrogen output was again observed, and amounted to a decrease of 17 per cent, essentially the same as in table 1.

To pursue the mechanism of this action of tolbutamide, the output of amino nitrogen and urea was determined. In this work the effect of tolbutamide was compared with that of insulin and of phenformin. The

TABLE 1

Effects of tolbutamide on nonprotein nitrogen (NPN) formation and on the conversion of C^{14}-glucose to CO_2 and glycogen in liver and diaphragm of rats

	NPN output		Glycogen		Glucose C^{14} to CO_2		Glucose C^{14} to glycogen	
	Mg. per 100 mg. liver per 3 hr.		Mg. per 100 mg.		μM per 100 mg. liver per 3 hr.			
	Control	Tolbutamide	Control	Tolbutamide	Control	Tolbutamide	Control	Tolbutamide
A. Liver slices in vitro*	.226†±.007 (16)‡	**.185**§±.008 (16)	.057±.010 (16)	.029±.006 (16)	1.52±.12 (16)	1.76±.14 (16)	1.68±.27 (16)	**0.70**±.11 (16)
B. Liver slices of rats pretreated with tolbutamide‖			.057±.010 (16)	.040±.008 (9)	1.52±.12 (16)	2.67±.37 (9)	1.68±.27 (16)	1.35±.29 (9)
C. Hemidiaphragms in vitro*	.336±.007 (11)	.309±.008 (11)	.166±.010 (11)	.128±.007 (11)	5.16±.62 (11)	6.20±.57 (11)	7.22±.71 (11)	6.00±.50 (11)
D. Hemidiaphragms of rats pretreated with tolbutamide‖			.166±.010 (11)	.163±.014 (9)	5.16±.62 (11)	4.16±.43 (9)	7.22±.71 (11)	7.35±.36 (9)

*Tolbutamide, 20 mg. per cent concentration in medium.
†Mean and standard error.
‡Number in parentheses is number of animals used.
§Bold type indicates significant difference from control ($P=<0.01$).
‖Tolbutamide, 10 mg. per 100 gm. rat once daily for three days before experiment.

TABLE 2

Effects of sodium mesoxalate on rat liver slices and hemidiaphragms

	NPN output		Glycogen		Glucose C^{14} to CO_2		Glucose C^{14} to glycogen	
	Mg. per 100 mg. liver per 3 hr.		Mg. per 100 mg.		μM per 100 mg. liver per 3 hr.			
	Control	Mesoxalate	Control	Mesoxalate	Control	Mesoxalate	Control	Mesoxalate
A. Liver slices in vitro*	.223†±.012 (11)‡	.228±.010 (11)	.043±.009 (11)	.040±.009 (11)	1.57±.24 (11)	1.82±.28 (11)	1.66±.26 (11)	1.82±.25 (11)
B. Liver slices of rats pretreated with mesoxalate§			.043±.009 (11)	.026±.003 (8)	1.57±.24 (11)	**3.86**‖±.61 (8)	1.66±.26 (11)	.80±.15 (8)
C. Hemidiaphragms in vitro*			.206±.018 (6)	.196±.011 (6)	4.67±.40 (6)	4.56±.60 (6)	5.23±.55 (6)	5.33±.40 (6)
D. Hemidiaphragms of rats pretreated with mesoxalate§			.206±.018 (6)	.220±.014 (8)	4.67±.40 (6)	4.35±.45 (8)	5.23±.55 (6)	6.98±.71 (8)

*Sodium mesoxalate, 1.0 mg. per cent concentration in medium.
†Mean and standard error.
‡Number in parentheses is number of animals used.
§Sodium mesoxalate, 0.5 mg./100 gm. rat per day for three days before experiment.
‖Bold type indicates significant difference from control ($P=<0.01$).

results provide a clear differentiation between the effects of tolbutamide and phenformin on the liver as tested here. The lack of any immediate effect of insulin on the liver slice is expected.[11] Tolbutamide reduces the amino acid output of the liver slice by an amount which accounts for approximately a quarter of the reduction in NPN output. Tolbutamide had no action on urea formation. In contrast, phenformin had no influence on the liberation of amino nitrogen, but strikingly reduced urea production. (Parenthetically, if the control

TABLE 3
Effect of tolbutamide on normal rat liver slices

Initial thirty minutes Mg. per 100 mg. liver per thirty minutes				Subsequent 3-hr. incubation Mg. per 100 mg. liver per 3 hr.			
Total N		NPN		Total N		NPN	
Control	Tolbutamide	Control	Tolbutamide	Control	Tolbutamide	Control	Tolbutamide
.178*±.017	.164±.011	.069±.008	.061±.003	.432±.095	.413±.020	.194±.003	**.161**†±.004

*Mean and standard error; six animals used. Tolbutamide, 20 mg. per cent concentration in medium.
†Bold type indicates significant difference from control (P=<0.01).

figures for urea production are expressed in micromoles, they agree with those of Stadie's[12] report.) When the livers of rats pretreated with phenformin were tested (last line, table 4), a significant depression in urea formation was also seen. This might represent the situation in the treated patient.

Since tolbutamide is a sulfonylurea and since urea as the end product of deamination might inhibit the reaction, the influence of an excess of urea in the medium was tested. In a concentration of 150 mg. per cent, urea caused no change in the nonprotein nitrogen output of liver slices. In two normal cats the fasting excretion of urinary nitrogen was measured, with and without three daily doses of 100 mg. of tolbutamide, but no difference was demonstrated. Even with this large dosage (at eight-hour intervals) the possible adjustment of protein metabolism between doses was recognized. Nitrogen balance has not been influenced in man by tolbutamide.[13]

Since a reduction of amino nitrogen output by the liver slice might be the result of inhibition of transaminases, the effect of tolbutamide on the liberation into the medium of two transaminases was determined. Table 5 presents the results which indicate that tolbutamide suppresses the ability of the liver to produce or liberate these enzymes. Control studies showed that tolbutamide in the concentrations employed in these determinations does not alter the activity of the enzymes themselves, and thus the results are due to an action on the tissue. Four determinations of the transaminase activity of homogenates of liver slices after incubation with or without tolbutamide failed to reveal any difference. These preliminary observations with homogenates differ from the inhibitory effect of tolbutamide found on alanine:α-ketoglutaric acid transamination, reported by Bornstein.[14] However, the results with the simple nonprotein nitrogenous substances studied and those on transaminase in media all support

TABLE 4
Effects of tolbutamide, insulin and phenformin on the output of three nitrogenous products by liver slices

Amino N—mg. per 100 mg. of liver slice per three hours		NPN—mg. per 100 mg. of liver slice per three hours		Urea N—mg. per 100 mg. of liver slice per three hours	
Control	Tolbutamide*	Control	Tolbutamide	Control	Tolbutamide
.043†±.002 (15)‡	**.035**§±.001 (15)	.180±.006 (15)	**.149**±.004 (15)	.037±.002 (22)	.035±.001 (22)
Control	Insulin‖	Control	Insulin	Control	Insulin
.045±.002 (12)	.039±.002 (12)	.188±.007 (12)	.164±.007 (12)	.037±.002 (13)	.035±.002 (13)
Control	Phenformin**	Control	Phenformin	Control	Phenformin
.039±.002 (9)	.048±.002 (9)	.188±.005 (9)	**.059**±.003 (9)	.040±.004 (6)	**.006**±.001 (6)
.042±.002 (36)	.048††±.002 (6)	.185±.004 (36)	.180±.006 (6)	.038±.002 (19)	**.023**±.001 (6)

*Tolbutamide 20 mg. per cent concentration in medium.
†Mean and standard error.
‡Figures in parentheses show number of rats used.
§Bold type indicates significant difference from control (P=<0.01).
‖0.1 unit per ml. in medium.
**Phenformin 0.5 mg. per ml. medium.
††In this series the rats were pretreated with phenformin (see text).

TABLE 5

Effect of tolbutamide on transaminase output of liver slices*

Glutamic oxalacetic transaminase			Glutamic pyruvic transaminase		
Units per 100 mg. liver slice per three hours					
Control	Tolbutamide	Tolbutamide effect	Control	Tolbutamide	Tolbutamide effect
20.9±1.6	16.9±1.6	—3.9±0.5†	4.2±0.5	3.3±0.4	—0.9±0.3‡

*Six rats used in each series. Tolbutamide, 20 mg. per cent concentration in medium. Mean and standard error, except tolbutamide effect which is difference between means and the standard error of the difference. Transaminase determinations performed by the William Pepper Laboratory, Hospital of the University of Pennsylvania.
†P=<.01.
‡P=<.05.

the concept that these hypoglycemic drugs act in a significant degree by the inhibition of gluconeogenesis.

In short, tolbutamide does not immediately alter the liberation of protein or urea but nevertheless causes an appreciable reduction of amino nitrogen and of other unknown components of nonprotein nitrogen. Phenformin does not act on amino nitrogen but inhibits urea formation (deamination).

Changes in CO_2 production and glycogen content. Table 1 shows that the glycogen content of both tissues was slightly reduced by tolbutamide and that the incorporation of glucose-C^{14} from the medium into glycogen was reduced in three of the four experimental conditions (table 1: A, B, C). The conversion of labeled glucose to CO_2 was slightly increased. Due to differences in methods, the results are not comparable to those of Ashmore et al.,[1] who found an increase in liver and muscle glycogen in fasted and fed normal rats one hour after pretreatment with tolbutamide. The increased utilization of carbohydrate by the liver, as measured by CO_2 production, agrees with the report of Recant and Fisher,[15] who inferred an increased utilization from the increased hepatic vein pyruvate.

Mesoxalate (table 2) did not alter glycogen content. In the liver, glucose utilization was increased as estimated by labeled CO_2 production, but the incorporation of glucose-C^{14} into glycogen followed no clear pattern.

COMMENT

Tolbutamide added in vitro decreases the output of nonprotein nitrogen by liver slices of normal rats. There is no change in the loss of protein or in the output of urea, but a reduction in amino nitrogen output accounts for about a quarter of this effect. Neither urea nor mesoxalate, added to the medium in the concentrations tested, have such an effect. The smaller but possibly valid effect on the nonprotein nitrogen output of diaphragm (table 1) suggests that this action is not limited to the liver but may apply to other tissues to a greater or lesser extent. If a reduction of nonprotein nitrogen of the order of 18 per cent occurred continually in patients treated with sulfonylureas, it should be a significant part of the metabolic effect. However, if this be so, it is not reflected in the nitrogen balance in our casual trials in the cat or in man.[13] On the other hand, a decreased hepatic nitrogen output is in accord with the increased incorporation of labeled glycine into liver protein reported by Recant and Fisher,[15] and with the diminished output of glucose by the liver after tolbutamide.[1] A level of tolbutamide in vitro, corresponding to that reported here, caused a 20 per cent depression in glucose production by rabbit liver slices.[16] The authors state that this supports the idea that there is an inhibition of glycogenolysis, but in table 1 above, liver glycogen and the incorporation of labeled glucose from the medium into glycogen were lowered, suggesting the primacy of an action on some component of protein metabolism. The ultimate meaning of these various findings requires further study.

Phenformin was found to behave differently. It caused a greater reduction in NPN output, a striking reduction in urea production and had no effect on amino nitrogen. These laboratory yardsticks clearly indicate differences between the effects of tolbutamide and phenformin on the liver slice. In the case of the rats pretreated with phenformin (last line, table 4), 10 mg. per 100 gm. were given twenty-four and two hours before the liver was taken. In these experiments, phenformin was not added in vitro.

In connection with other studies on these drugs, it is clear that, except for a few pretreated animals, the effects reported above took place in the presence of whatever insulin was bound to the tissues but in the absence of added insulin secretion.

SUMMARY

The addition of tolbutamide to the incubating medium caused a reduction in the liberation of nonprotein nitrogen by liver slices and to a lesser degree by dia-

phragms of normal rats. There was no change in the output of protein or urea by liver slices, but that of amino nitrogen was reduced, as was the transaminase activity of the medium. Sodium mesoxalate had no such action on nitrogen output. The simultaneous changes in glycogen content, conversion of labeled glucose in medium to CO_2, and to glycogen, were recorded but could not be clearly related to the changes in nitrogen metabolism. Phenformin caused a greater reduction in the output of nonprotein and urea nitrogen, but had no effect on amino nitrogen. Both tolbutamide and phenformin affect the liver but do so in different ways as measured by nitrogen metabolism in vitro.

SUMMARIO IN INTERLINGUA

Effectos in Vitro de Tolbutamida, Mesoxalato, e Phenformina Super le Liberation de Nitrogeno per Trenchos de Hepate de Ratto

Le addition de tolbutamida al terreno de incubation causava un reduction in le liberation de nitrogeno non ligate a proteina ab trenchos de hepate de rattos normal e, minus pronunciatemente, ab le diaphragma de tal animales. Esseva notate nulle alteration in le rendimento de proteina o de urea per le trenchos de hepate, sed illo de amino-nitrogeno esseva reducite, e un reduction esseva etiam constatate in le activitate transaminasic del terreno. Mesoxalato de natrium non habeva iste effecto in le rendimento de nitrogeno. Le simultanee alterationes del contento de glycogeno e le conversion de marcate glucosa del terreno in CO_2 e glycogeno esseva registrate sed non poteva esser relationate clarmente al alterationes del metabolismo de nitrogeno. Phenformina causava un plus grande reduction in le rendimento de nitrogeno non ligate a proteina e de nitrogeno de urea sed habeva nulle effecto super le nitrogeno de aminas. Tanto tolbutamida como etiam phenformina affice le hepate, sed in tanto que le metabolismo de nitrogeno in vitro permitte mesurar lo, le maniera in que ille effecto occurre es differente in le duo casos.

ACKNOWLEDGMENT

The authors are grateful to Miss Edythe Gershman of the Pepper Laboratory, Hospital of the University of Pennsylvania, for the determinations of transaminase activity reported herein. The suggestions of Dr. M. E. Krahl of the University of Chicago were most helpful.

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Peroral Jejunal Biopsy in a Patient with Diabetic Diarrhea

A Case Report

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The "diarrhea of diabetes" was first described in 1936 by Bargen et al.[1] It is characterized by frequent, watery stools usually nocturnal in occurrence. Intermittency of symptoms and fecal incontinence further delineate this form of diarrhea. It is generally associated with diabetes of long duration which has been inadequately controlled. An associated peripheral neuropathy is common, and, indeed, it is accepted that diabetic diarrhea is merely another manifestation of the neuropathy of diabetes. Diabetic diarrhea usually is classified along with the neurogenic bladder, impotency, and postural hypotension as a form of "autonomic neuropathy."

Berge et al.[2] in a careful study of autopsy specimens from patients with diabetic diarrhea were unable to find any significant gross or microscopic abnormalities in the gastrointestinal tract. Ellenberg and Bookman[3] have recorded an abnormal peroral jejunal biopsy in a patient with a malabsorption syndrome and diabetes. Steatorrhea has also been associated with diabetic neuropathy.[4] The jejunal mucosal pattern in a patient with diabetic diarrhea is reported herein.

CASE REPORT

C.K. (HFH No. 983553), a forty-five-year-old white mechanic, was referred to the Henry Ford Hospital because of uncontrolled diabetes and chronic diarrhea. He had known of the diabetes for nineteen years, but had been under medical supervision only sporadically during this time. Insulin therapy had been used throughout. One year prior to admission, the patient noted the onset of diarrhea. This consisted of three to five watery movements, usually beginning in the early morning hours and ending around breakfast time. Involuntary loss of feces occurred several times. He also noted daytime nausea and intermittent peri-umbilical pain of a crampy nature which was postprandial in timing. No vomiting occurred. Intake of food relieved this distress. His admission insulin prescription was 8 units of Crystalline Zinc Insulin (CZI) and 20 units of Semilente insulin combined every morning. He also took 75 mg. of phenformin (DBI) daily in divided doses. The latter had been used for only one month prior to admission. He complained of being sexually impotent.

Weight on admission was 144 pounds and height was 70 inches. There was no fever. Blood pressure was 130/80 recumbent and 80/60 standing. Grade III diabetic retinopathy (microaneurysms, punctate hemorrhages and waxy exudates) was present. Physical examination of the heart, lungs and abdomen was negative. Neurologic examination showed decreased vibratory sensation and hypoactive deep tendon reflexes in both legs. The skin lesions of psoriasis were present.

Routine laboratory work was normal except for a trace of albumin in the urine, four-plus glycosuria, and a serum urea nitrogen of 32 mg. per 100 ml. (normal in our laboratory is 10-23 mg. per 100 ml.). Gastric acidity was normal and a stool examination was negative for ova and parasites. Proctoscopic examination to 24 cm. was negative, as was a complete radiographic study of the stomach, small bowel and colon. No delayed emptying of the stomach or malabsorption pattern in the small bowel was noted. A normal fasting serum carotene of 122 μg. per 100 ml. was found. A jejunal mucosal biopsy* using the Crosby capsule technic was obtained.[5] Careful microscopic study of an adequate specimen revealed normal jejunal mucosa (figure 1).

Four months following discharge from the hospital the diarrhea had abated almost completely. The patient reported one to three watery stools every seven to ten days. A weight gain of eighteen pounds had been effected. Nocturnal pain in both feet is the major problem at this time.

DISCUSSION

This patient presented the usual criteria for diabetic diarrhea; namely, diabetes of long duration, intermittent fecal incontinence, other evidences of peripheral and autonomic neuropathy, and a normal medical work-up of the entire gastrointestinal tract. This patient had diabetic triopathy (neuropathy, nephropathy, and retinopathy) as described by Root, et al.[6] Therapeutic advice included careful control of his diabetes, a bowel rest program (bland low residue diet, avoidance of stimulants, antispasmodics, etc.), and supplementary vitamin B_1 and B_{12}. He was discharged on 26 units of Lente insulin every morning and 8 units at bedtime.

The association of diabetes and disorders of the gastro-

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* We are indebted to Dr. William S. Haubrich for performance of the jejunal biopsy.

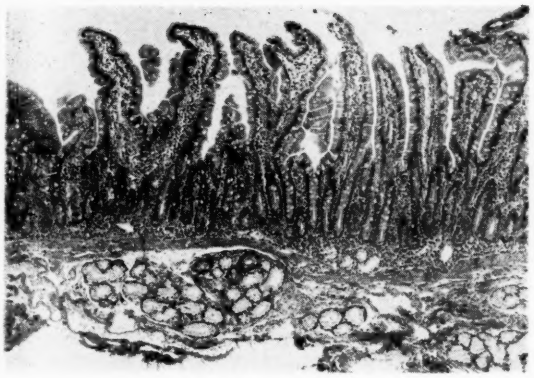

FIG. 1. Normal jejunal mucosa obtained perorally from a patient with diabetic diarrhea.

intestinal tract is well known. Beside diabetic diarrhea, Kassander[7] accumulated twenty-seven cases of delayed gastric emptying (gastroparesis diabeticorum) in a group of patients with diabetes. Diabetes and a malabsorption syndrome have occurred concomitantly. However, in the diabetic patient, simple nocturnal diarrhea is probably the most common symptom complex associated with neuropathy. The causal relationship between diabetes and these gastrointestinal manifestations is always difficult to prove. It is so easy to blame diabetes for symptoms when other obvious causes are not apparent, yet the patient with diabetes might develop coincidentally a chronic diarrhea or a malabsorption syndrome just as a person without diabetes might do. However, considerable experience by many authors makes the relationship of diabetes and nocturnal diarrhea more than coincidental. The same cannot be said of diabetes and the malabsorption syndrome. In our clinic only one instance of the latter combination has been seen. The jejunal mucosal biopsy in this patient was abnormal, showing clubbing and flattening of the villi.

Our patient has chronic nocturnal diarrhea. He also has sexual impotency and postural hypotension. We relate all three to a neuropathy involving autonomic pathways. Further jejunal mucosal biopsies are planned in patients who fulfill the criteria for diabetic diarrhea. The state of the intestinal mucosa in such patients during life should be established. Based on previous postmortem studies, one might expect normal mucosal biopsies in patients with simple nocturnal diarrhea.

SUMMARY

A case of chronic nocturnal diarrhea in a patient with diabetic neuropathy is presented. Normal jejunal mucosa, obtained perorally, was found on biopsy examination.

SUMMARIO IN INTERLINGUA

Biopsia Peroral del Jejuno in un Patiente con Diarrhea Diabetic: Reporto de un Caso

Es presentate un caso de chronic diarrhea nocturne in un patiente con neuropathia diabetic. Le examine de un specimen bioptic de mucosa jejunal, obtenite per via oral, esseva normal.

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Ultra-micro Sugar Determinations Using 2,9-dimethyl-1,10-phenanthroline Hydrochloride (Neocuproine)

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This communication is concerned with a chemical method for the determination of blood sugar by an "ultra" micro technic on blood volumes as little as 0.01 to 0.03 ml. The need for such a method and its development occurred during experiments on small diabetic animals (guinea pigs) when difficulties were encountered in collecting as little as 0.05 to 0.1 ml. of blood. Although the literature abounds in reports on technics for micro blood sugar determinations, no copper reduction method has yet been reported to be applicable to these small quantities of blood.[1-6,14]

This method follows that originally described by Folin and Wu, with two important modifications, namely, a twentyfold reduction in the amount of crystalline copper sulfate used to prepare the alkaline copper solution, and a replacement of the phosphomolybdic acid by a solution of neocuproine.

The use of 2,2′-diquinoline (cuproine) for the detection of the cuprous ion was described by Hoste in 1950.[6] Later reports by Smith, Gahler and others showed that 2,9-dimethyl-1,10-phenanthroline (neocuproine) was highly specific for the cuprous ion, and that the presence of fifty-six other metallic ions, including the cupric ion, did not interfere in the reaction of neocuproine with cuprous ion.[7-9] It was further shown that the final color was very stable over a *p*H range of 3 to 10, and that the presence of chloride, tartrate, citrate, acetate, phosphate and several other negative radicals did not interfere with the final color development. The availability of the water soluble neocuproine hydrochloride salt suggested that it might serve as a replacement for the various "molybdic acids" used in the copper reduction sugar methods.

In order to validate the use of an "ultra" micro blood sugar technic, comparison with the macro Folin-Wu blood sugar method was made.

Reagents:

(A) Alkaline dilute copper solution.
Dissolve 40 gm. of anhydrous sodium carbonate in about 400 ml. of distilled water in a liter flask. Add 7.5 gm. of tartaric acid and, when dissolved, add 0.225 gm. of crystalline copper sulfate ($CuSO_4 \cdot 5H_2O$). Mix and dilute to the liter mark. (This solution contains about 58 μg. of copper per milliliter.) Alternately, the Folin-Wu alkaline copper solution may be diluted twentyfold with a solution containing 40 gm. of sodium carbonate and 7.5 gm. of tartaric acid per liter.

(B) 0.4 neocuproine solution.
Dissolve 0.4 gm. of neocuproine hydrochloride* in 100 ml. of distilled water. This solution is colorless and stable for six months or longer, provided it does not become contaminated with cuprous ions.

(C) 0.0417 (1/24) normal sulfuric acid.
Dilute 1/12 normal sulfuric acid (as proposed by Haden)[10] with an equal volume of water.

(D) 10 per cent sodium tungstate W/V.

Preparation of 1:101 blood filtrate:

The blood sample 0.02 ml. (20 lambda) is added to 1.95 ml. of 0.0417 *N* sulfuric acid with careful rinsing of the blood pipette,† and immediate shaking of the tube. Then 0.05 ml. of 10 per cent sodium tungstate is added, mixed, and allowed to stand for ten minutes. Upon centrifugation a clear supernatant solution is obtained.

Procedure:

1. One milliliter of 1:101 filtrate (or any equivalent amount of solution containing from 5 to 30 μg. of glucose) is placed in a 10-ml. Sunderman sugar tube.‡ (See footnote on page 61.) Volumes other than 1 ml. are made up to 1 ml. with distilled water.

2. A blank of distilled water and glucose standards (10, 20 and 30 μg.) are similarly prepared.

From the Laboratory of The Faulkner Hospital, Boston, Massachusetts.

*The neocuproine hydrochloride (2,9-dimethyl-1,10-phenanthroline hydrochloride) was obtained upon request from G. Frederick Smith Chemical Company, Columbus, Ohio.

†A reliable 20 cmm. Sahli hemoglobin pipette, or a 25 lambda pipette calibrated in 5 lambda may be used.

3. Two ml. of the alkaline dilute copper solution A are then added to each tube.

4. Add 0.3 ml. of the 0.4 per cent neocuproine (solution B).

5. The sugar tubes are then placed in a vigorously boiling water bath for four to six minutes. Whatever time is chosen should be adhered to for all of the tubes in the series, including the blank. We have routinely used a time of five minutes.

6. The tubes are removed from the water bath and cooled sufficiently to permit handling, and then diluted to the 10 ml. mark with distilled water. The tubes are mixed by inversion several times.

7. Using a Coleman model 6A spectrophotometer with 19 x 150 mm. cuvettes, the blank is set at zero optical density at 450 mu.

8. The standards are sufficiently reproducible to construct a permanent calibration chart from which to read the unknowns.

Calculations:

According to the above outlined procedure, the glucose standards of 10, 20 and 30 μg. (because of the 1:101 dilution) are equivalent to 101, 202, and 303 mg. of glucose per 100 ml. of whole blood.

RESULTS

Figure 1 (upper curve) illustrates the relationship of the optical densities to various glucose concentrations. The curve is linear from 0 to 30 μg. of glucose. The lower curve of figure 1 for hydroxylamine reduced copper (0 to 40 μg.) shows that 20 μg. of reduced copper is equivalent to 14.6 μg. of glucose. This reduced copper to glucose relationship is predictable by extrapolating the glucose values (equivalent to 0.1 to 4.0 mg. of reduced copper) obtained by Somogyi[11] and others.[12]

Figure 2 illustrates that all of the whole blood sugar values obtained by the method presented agree within 10 per cent with those obtained by the classical Folin-Wu method. In fact, the average error of fifty-one analyses was 5.1 per cent. The coefficient of correlation between these methods was 0.96, as shown in figure 2. The error of duplicate analysis by the proposed method was less than 5 per cent, which is about the same as that found for duplicate macro Folin-Wu sugar analyses.[12] Duplicate analyses of the glucose standards were within 3 per cent.

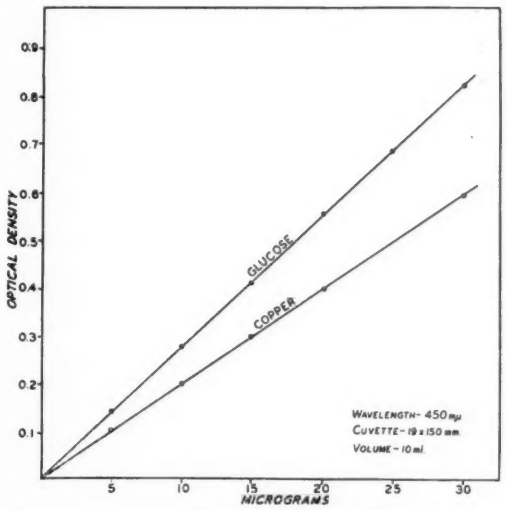

FIG. 1. Illustrates the optical densities obtained for glucose and reduced copper of similar concentrations. The glucose determinations were made according to the procedure described in the text. The copper sulfate standards were reduced with hydroxylamine hydrochloride, and the pH adjusted to 9.8 with an alkaline-tartrate solution before the final cuprous-neocuproine was developed by the addition of the neocuproine solution.

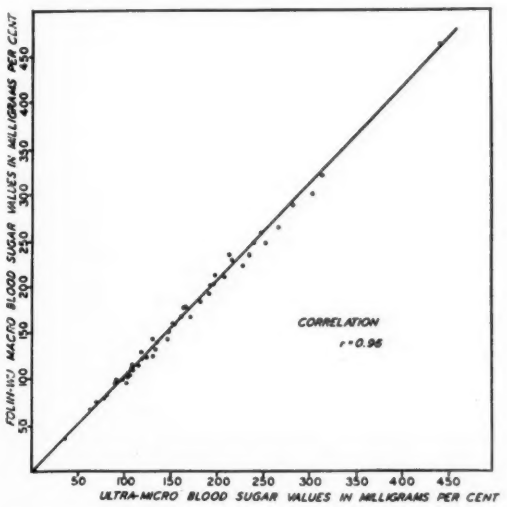

FIG. 2. The sugar values of fifty-one whole blood samples were obtained by the classical macro Folin-Wu method and the ultra-micro neocuproine method as proposed. A significant coefficient of correlation of 0.96 was obtained when the values for the two methods were compared.

‡ Where it is inconvenient to obtain calibrated 10 ml. sugar tubes, the ordinary 25 ml. Folin sugar tube may be used by the addition of 0.7 ml. of distilled water to all the sugar tubes so that the final volumes of the tubes in Step #4 is 4 ml. In Step #6, the addition of 6.0 ml. of distilled water will yield a volume sufficiently close to 10 ml. so that reproducible results are obtained.

Neocuproine could be incorporated into the alkaline dilute copper solution for each day's analyses without adverse effects. The neocuproine solution added to alkaline tartrate solution (containing no copper) and boiled for ten to twenty minutes did not produce color. Sufficient amounts of neocuproine remained to detect several hundred micrograms of reduced copper.

When several sets of 20 μg. glucose standards with their respective blanks were boiled for periods from two to eight minutes, it was found that the three- to eight-minute boiling periods did not alter the amount of copper reduced by more than 2 per cent. The two-minute boiling period of the standard with its respective blank reduced 68 per cent of the theoretical amount of copper. The blanks for the above corresponding boiling periods (three, four, five, six and eight minutes) were found to be equivalent to 3.3, 5.5, 6.8, 7.4 and 7.9 μg. of glucose respectively. While this amount of copper reduction in the blanks was considered slightly more than that expected for the auto-oxidation-reduction of alkaline copper solutions, it nonetheless did not affect the reproducibility of the standards when a blank was included.

When the alkaline dilute copper solution was made to contain varying amounts of copper sulfate (100 to 300 μg. of copper) and treated as a blank according to the procedure, it was found that an average increase equivalent to 0.2 μg. of glucose occurred for every 10 μg. of copper. Thus a 10 per cent error in pipetting the alkaline dilute copper solution would result in less than 2 per cent error.

Changing the final *pH* (9.8) to various *pH* values (5.0 to 10.0) by dilute acid or buffer did not affect the colorimetric readings, but served only to release carbon dioxide and make colorimetric readings more difficult. The final orange-red- neocuproine-cuprous complex was stable for ten hours or more in diffuse sunlight at room temperature.

COMMENTS

The only objection to the use of neocuproine hydrochloride as a color reagent for sugar analyses was that the blanks were slightly greater than anticipated. On the other hand, neocuproine was found to be about thirty times more sensitive to the cuprous ion than Folin's phosphomolybdic acid, and a more careful consideration would reveal that an equivalent amount of auto-oxidation-reduction of the alkaline copper solution also occurs in the classical Folin-Wu method.

The above procedure has been adapted to the Klett and Evelyn colorimeters (440 filters), with slightly higher optical density readings for the glucose standards by the latter instrument. Hence the effective range with the Evelyn colorimeter was from 4 to 20 μg. of glucose. Further reduction of the final reading volume from 10 ml. to 6 ml. permitted determinations on samples containing from 2 to 15 μg. of glucose.

Use of neocuproine in other copper reduction methods (Somogyi, Benedict) tentatively appears feasible.

SUMMARIO IN INTERLINGUA

Ultramicrodeterminationes de Sucro Sanguinee con le Uso de Hydrochloruro de 2,9-Dimethyl-1,10-Phenanthrolina (Neocuproine)

Le presente reporto es concernite con un methodo pro le determination de sucro sanguinee per medio de un ultramicrotechnica que require volumines de sanguine de solmente 0,01 a 0,03 ml. Ben que le litteratura abunda in reportos de technicas pro le microdetermination de sucro sanguinee, nulle methodo a reduction de cupro ha essite reportate con applicabilitate a specimens del micre magnitudes hic considerate.

Le methodo seque illo originalmente describite per Folin e Wu, con duo importante modificationes, i.e. (1) un reduction vintuple in le quantitate de crystallin sulfato de cupro usate in preparar le solution alcalin de cupro e (2) le substitution de un solution de neocuproina pro acido phosphomolybdic.

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Special Article

Comments on the Cortisone-Glucose Tolerance Test

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In 1948 it was reported[1] that a state of temporary diabetes mellitus could be induced in normal humans by administration of pituitary adrenocorticotropin. Persons resistant to the diabetogenic action of ACTH were similarly resistant when cortisone was given, while those susceptible were sensitive to the diabetogenic effect of either substance.[2]

In 1950 work was begun for the purpose of seeking answers to the following questions: (1) Can the susceptibility for the development of future diabetes be detected in presently nondiabetic relatives of diabetic patients and (2) Can the diabetogenic activity of ACTH or adrenal glucocorticoids sufficiently enhance the sensitivity of the standard oral glucose tolerance test that future diabetics might be detected earlier?

In 1954 a standardized procedure, the so-called cortisone-glucose tolerance test, was described as a possible approach to the prediction of diabetes mellitus.[3] It was reported that this test separates into two groups the presently nondiabetic relatives (children, siblings or parents) of diabetic patients. One group with a "positive response" to the test responded as did the majority of mildly diabetic patients while the other exhibiting a "negative response" reacted as did the majority of control individuals selected because they had no family history of diabetes. It was concluded that the value of the test would be determined by time and careful follow-up studies regarding the incidence of future diabetes in each of the two groups. In 1955, 1958 and 1959 further experience with the cortisone-glucose tolerance test in a progressively larger series of individuals was reported.[4-6] Follow-up studies were presented,[5-7] which showed that 25 per cent of forty subjects with a "positive response" to the initial cortisone-glucose tolerance test had developed diabetes and that another 10 per cent had developed "probable diabetes." On the other hand, of fifty-nine subjects with a "negative response" to the cortisone-glucose tolerance test only one had developed diabetes. Thus, the cortisone-glucose tolerance test was felt to be promising as an initial step in the quest for earlier detection of the diabetic state.[5,6]

We still regard the cortisone-glucose tolerance test in the same light. Although it seems to be emerging as a diagnostic procedure more sensitive than the glucose tolerance test, it is still in the investigative stage. Only follow-up studies over many more years can determine how accurate it is in predicting future clinical diabetes. But prediction is an empty goal! The purpose of earlier detection of disease is to find means of improving its treatment or, better, of preventing its development. For us, the cortisone-glucose tolerance test is a research tool. It has enabled us to observe and study the diabetic state before it was detectable by conventional methods. This is our major concern—to find the future diabetic and to study him. That some future diabetics may at present give a negative cortisone-glucose tolerance test is an entirely different problem which also requires careful study.

Several studies evaluating a steroid-modified glucose tolerance test for the detection of prediabetes have been reported recently. Some of these reports have confirmed the findings and conclusions mentioned above while others have questioned the usefulness and validity of such a procedure. The purpose of this communication is to delineate the areas of agreement and disagreement in the published reports with the hope of dispelling misunderstanding and confusion.

Results of some of the recent studies which report the use of steroid-glucose tolerance tests cannot be compared with those originally published since they employ

(1) capillary blood rather than venous blood[8-11]
(2) different blood sugar methods[8,10-13]
(3) different loading doses of glucose[8-14]
(4) different routes of administration of glucose[12]
(5) no standard dietary preparation preceding the glucose tolerance test[9,12,15]
(6) different steroids[8,9,14]
(7) different dosages of steroids[8,9,12,14]
(8) different timing of administration of steroids[8,9,12]
(9) different criteria for the interpretation of the glucose tolerance test and the steroid-glucose tolerance test.[10-12,14]

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Nevertheless, agreement with the general conclusions mentioned above can be found in several reports. Nusimovitch[14] found that 28 per cent of healthy relatives of diabetic patients had positive responses to a prednisolone-glucose tolerance test while the same response was obtained in only 2 per cent of control patients. This incidence is very similar to that reported (24 per cent to 27 per cent) in the studies quoted above.[3-6,16] Goto and his associates[5] concluded that the diabetogenic action of prednisolone was of value in making a distinction between normal or nondiabetic patients on the one hand, and relatives of diabetic patients on the other hand; and, that the prednisolone-glucose tolerance test is useful in the detection of the prediabetic state. In subjects under the age of forty, Sanders,[6] using a prednisolone-glucose tolerance test, also confirmed the findings of a greater decrease in carbohydrate tolerance in relatives of diabetic patients than in normal subjects. That a prediabetic state was indeed detected was suggested by the fact that the fourteen-year-old nondiabetic twin of a diabetic, the former exhibiting a markedly positive response to the prednisolone-fortified glucose tolerance test, developed frank diabetes himself one year later. Three other "positive reactors" under the age of forty have subsequently developed diabetes.[6] Utilizing an intravenous glucose tolerance test Duncan found no change in carbohydrate tolerance following administration of cortisone to normal subjects.[12] In patients with mild diabetes mellitus and in patients with latent diabetes there was significant decrease in glucose tolerance. Nineteen patients were suspected of being in a prediabetic state. In nine of these significant impairment of glucose tolerance was induced by cortisone, and of these nine subjects two became frankly diabetic at a later date.[12]

A critical examination of some of the reports which have questioned the usefulness of the cortisone-glucose tolerance test or similar tests for early detection of diabetes mellitus discloses

(1) that some of the data reported are not comparable to those presented in the original studies
(2) that there has been considerable misinterpretation of the conclusions reached in the original reports or that implications are attributed to that work which were neither expressed nor intended.

The following points seem worthy of detailed discussion:

Performance and evaluation of the glucose tolerance test. Criteria for the interpretation of the standard oral glucose tolerance test have been reported.[3,5-7] It has been emphasized that an evaluation of blood sugar levels one, one and a half and two hours after administration of glucose is necessary to determine whether abnormality of carbohydrate utilization exists.

West[15] studied glucose tolerance and steroid-glucose tolerance in subjects both of whose parents were diabetic. In these studies the assumption was made that 100 per cent of the offspring of two diabetic parents should have or will develop diabetes mellitus if all of the individuals live to old age. The mean age of the individuals in West's series was thirty-seven years. After administration of a standard glucose load the diagnosis of diabetes was made or excluded by the use of a *single* two-hour blood sugar level set at 115 mg./100 ml. Although the two-hour postprandial blood sugar level is an excellent screening procedure for diabetes mellitus it cannot be employed as a diagnostic procedure if only slightly elevated. It is impossible to evaluate whether such a blood sugar level is preceded by a diagnostic peak level of 160 mg./100 ml. or above, or whether it is a part of a relatively flat blood sugar curve in which the two-hour level is in the same range or actually higher than earlier levels during the procedure.[7] Secondly, one cannot rule out a nondiabetic "rebound curve" by the use of a single two-hour blood sugar level.[6] Such a blood sugar curve could not be considered diagnostic of diabetes mellitus even though the two-hour level is slightly elevated.[7] On the basis of these considerations the diagnosis of diabetes cannot be made in four of eight individuals in West's series in whom diabetes was said to have been discovered. (It is interesting to note that three of these four individuals had positive cortisone-glucose tolerance tests.) Thus, the author has little justification for his claim that 29 per cent of the offspring of two diabetic parents in his series had diabetes. West states that interindividual variability in glucose levels was great in his experience. This is difficult to evaluate when one uses for comparison only one blood sugar level rather than a series of levels after administration of glucose. Similarly, he compounds his interpretative difficulties when he attempts to compare the results of the cortisone-glucose tolerance test with those of the standard glucose tolerance test on the basis of one two-hour blood sugar level.

Does the cortisone-glucose tolerance test measure genetic susceptibility to diabetes? In thirty nondiabetic subjects (mean age thirty-seven years) both of whose parents were diabetic West did not find an increased incidence of abnormal cortisone-glucose tolerance tests.[15] This was true even when the results were compared with those of a control group without a history of diabetes mellitus.

In our own studies we have found a 37 per cent incidence of "positive responses" among forty-six subjects

both of whose parents are diabetic.[16] This is to be compared with a 24 per cent incidence for the remainder of the nondiabetic relatives of diabetics, many of whom also had a bilateral (not involving both parents) family history of diabetes. The increased incidence of a "positive response" among nondiabetic members of families with diabetes mellitus or among individuals suspected of being prediabetic as reported by Goto,[8] Sanders,[9] Duncan,[12] and Nusimovitch[14] was mentioned above. But on the basis of his findings West concludes that the cortisone-glucose tolerance test is unlikely to "identify with any appreciable accuracy the presence of genetic susceptibility to diabetes."[15]

Jackson[10,11] likewise considers a negative cortisone-glucose tolerance test in mothers of large or stillborn babies or in children with a bilateral family history of diabetes as a "false negative test" since it is known that many of such people will eventually develop diabetes. It has never been implied that the test is capable of detecting *genetic* susceptibility to diabetes. The cortisone-glucose tolerance test should be regarded as a more sensitive test than the standard glucose tolerance test in the early detection of abnormal carbohydrate metabolism during the natural history of the disease called diabetes. This does not imply that one should expect this test to be positive for a lifetime in subjects destined to become diabetic in the future. Were this the case one could simply screen out all of our future diabetics during their first week of life. Although a "positive cortisone-glucose test" in certain nondiabetic individuals can be interpreted as indicating increased susceptibility to future development of diabetes, a negative test does not rule out a predisposition to diabetes and should not be considered as a "falsely negative test." The cortisone-glucose tolerance test is not positive in all stages of the prediabetic period and this has not been claimed for it. For instance, production of a large baby may be an earlier indication of prediabetes than a positive cortisone-glucose tolerance test. In our own experience[16] the cortisone-glucose tolerance test has been positive in only 50 per cent of a small series of women who had given birth to babies weighing ten pounds or more. Nevertheless, this incidence is twice that found in nondiabetic relatives of diabetics and thirteen times that observed in people with no family history of diabetes. The fact that there are rare circumstances which indicate the presence of prediabetes before it can be detected by a steroid-modified-glucose tolerance test does not detract from the potential usefulness of the test. The majority of individuals with a family history of diabetes mellitus do not have suggestive evidence of prediabetes on obstetrical or genetic (both parents diabetic or an identical twin with diabetes) grounds. For lack of a better way, a positive cortisone-glucose tolerance test is likely to be their earliest indication of oncoming diabetes mellitus.

Age. Jackson,[10,11] West[15] and Sanders[9] have indicated that a positive cortisone-glucose tolerance test is found with greater frequency in older people (over age forty to forty-five) than in younger ones. This finding needs careful evaluation. It may indicate no more than a greater susceptibility to diabetes with advancing age. However, if cortisone should consistently produce a higher rate of "positive responses" in older individuals without a family history of diabetes, different criteria for abnormality would have to be defined for the older age group.

Although the age range in our own series has extended to sixty-seven years, the great majority of subjects have been below the age of forty-five with a mean age of thirty years. The mean age of individuals with a negative cortisone-glucose tolerance test is twenty-eight years (range seven to sixty-two years). The mean age of patients with a positive cortisone-glucose tolerance test is thirty-six years (range seven to sixty-seven years). This is almost the same mean age as that reported by West in nondiabetic subjects (mean age thirty-seven) whose parents are both diabetic and in whom he found no increased incidence of "positive response" to the test. Thus, West's suggestion[15] that the difference of response between our "positive" and "negative" responders was due to age alone is not acceptable.

There is no unanimity of opinion as to the effect of age on glucose tolerance. One needs to consider decreased mobility, increased bed rest, chronic illness, decreased food intake, and other factors frequently operative in older people, which, per se, may affect glucose tolerance. Although conclusive studies on the effects of old age alone on carbohydrate tolerance are not available, the majority of investigators appear to accept as fact a physiological decline of carbohydrate tolerance with advancing age. If this is true then one would expect to find an increased incidence of positive responses to the cortisone-glucose tolerance test with advancing age in nondiabetic individuals without a predisposition to diabetes. Our own data are insufficient to answer this question. Further data are needed before one can decide in what way criteria for the interpretation of the cortisone-glucose tolerance test will have to be changed to be meaningful in the older age group.

Evaluation of the effect of age on cortisone-modified glucose tolerance needs to be carried out in individuals with normal standard glucose tolerance tests. Sanders[9]

control patients, as well as individuals with a family history of diabetes, over the age of forty (mean age sixty-two and forty-eight years, respectively) had composite standard glucose tolerance tests which exceeded limits which we define as normal. One cannot compare the incidence of a "positive response" in a group of individuals whose standard glucose tolerance tests are abnormal with a group whose standard glucose tolerance tests fall clearly into the normal range.

If there is a gradual physiological loss of carbohydrate tolerance with advancing age one would expect increased carbohydrate tolerance in the young. Diabetes is a disease which we recognize with greatest frequency in middle age. Since the cortisone-glucose tolerance test very likely does not spread its detective capacity over a span of decades, one would expect to find a lesser incidence of positive responses in children, including those with a family history of diabetes mellitus. However, where the prediabetic period is telescoped so that diabetes is recognized in the early years of life the cortisone-glucose tolerance test has been found to be positive in early life and prior to the development of diagnosed diabetes.

As evidence that young individuals are less responsive to cortisone than older ones, West cites his finding that hydrocortisone administration to five young healthy subjects for one week caused no loss of carbohydrate tolerance.[15] One cannot compare administration of steroid for a week with what occurs after two doses six and one-half hours apart as in the cortisone-glucose tolerance test. After chronic administration of cortisone one is less likely to differentiate between the normal and the prediabetic individual. An individual with a positive response to the cortisone-glucose tolerance test may have a negative response after several days of administration of steroids.[10] It is likely that the defect being detected by the cortisone-glucose tolerance test is an inability to increase insulin secretion promptly in response to cortisone rather than an inability to do so eventually.

Obesity. German[13] found no difference in the results of the cortisone-glucose tolerance test in forty obese and forty nonobese individuals without a family history of diabetes. West[15] cites this finding as further evidence that "prediabetic" individuals are not unusually responsive to the hyperglycemic effects of cortisone. This statement is based on the fact that diabetes is much more likely to develop in the obese. However, obesity, per se, is not to be construed as a prediabetic state. Obesity is a precipitating or inciting factor only in those individuals genetically predisposed to diabetes. German's obese patients had no family history of diabetes. Furthermore, obese people whose carbohydrate tolerance does not diminish under the strain of obesity are almost invariably those with no family history of diabetes. Thus, German's obese group with normal carbohydrate tolerance would be less likely to contain prediabetics than his group at normal weight.

Cortisone-glucose tolerance in mild diabetes. An objection raised to the specificity of the steroid-glucose tolerance test is that not all patients with mild diabetes show further loss of carbohydrate tolerance during the cortisone-glucose tolerance test.[10,11,15] In our own experience approximately 85 per cent of mildly diabetic and "probable diabetic" patients have a further significant decrease in carbohydrate tolerance after administration of cortisone. This was indicated in our original publication in 1954[3] and in subsequent reports.[5,6] This augmented incidence of decrease in carbohydrate tolerance in diabetic individuals after administration of cortisone is more than three times higher than that found in nondiabetic relatives of diabetic patients, and seventeen times higher than in control subjects in our experience. In patients with mild or latent diabetes mellitus Duncan[12] reported significant decrease in glucose tolerance after administration of cortisone. Goto et al.[8] found 100 per cent incidence of significant decrease in carbohydrate tolerance in twenty mildly diabetic patients in whom a steroid-glucose tolerance test was done. Thus, Goto concluded that the prednisolone-glucose tolerance test was useful in the interpretation of borderline standard glucose tolerance tests. On the other hand, West[15] states that "subjects who already exhibited slight impairment of tolerance showed no evidence of increased responsiveness to cortisone." Examination of West's data indicates that eleven of twenty-six subjects thus identified had two-hour blood sugar levels under 120 mg./100 ml. by the Somogyi-Nelson technic, or under 140 mg./100 ml. by the Folin-Wu technic. They cannot be regarded as having "impairment of carbohydrate tolerance" with these single, two-hour blood sugar levels as indicated earlier. Furthermore, many of these individuals showed large increases in blood sugar levels after cortisone.

Pregnancy. Jackson[11] has questioned appropriately whether a positive response to the cortisone-glucose tolerance test obtained *during* pregnancy is indicative of the presence of the prediabetic state. We have not put forward any such suggestion since in our experience the cortisone-glucose tolerance test has yielded a positive response in a very large proportion of patients during the latter stages of pregnancy.[10] We do not know by what mechanism late pregnancy induces increased sensitivity to the hyperglycemic effect of a test dose of cortisone. It seems possible that in this complex metabolic

state pancreatic reserve is sufficiently taxed so that some normal women react temporarily to the cortisone-glucose tolerance test in the same way that prediabetic women do. Jackson[11] wonders why the test dose of cortisone is more hyperglycemia-provoking in pregnancy. He points out that plasma 17-hydroxycorticoids are already elevated and that this state of affairs would have been expected, per se, to have resulted in decreased carbohydrate tolerance, if exogenous cortisone can induce such a decrease. Why the difference between endogenous and exogenous glucocorticoid activity? The answer probably resides in the demonstration that the principal increase in plasma 17-hydroxycorticoids during pregnancy is in the protein bound fraction rather than in the biologically active, free fraction.[17,18] Thus, it seems likely that the strain upon the pancreatic beta cells which exists in late pregnancy is exerted by an influence other than excessive glucocorticoid activity. This area is worthy of intensive investigation.

Is the cortisone-glucose tolerance test yielding any information? We believe it is, within the limits for which it was designed—but it is still too early to be certain. Our follow-up studies in more than one hundred subjects observed for one to seven years after initial testing are encouraging but the numbers are still small.[5-7,16] The results of a few more follow-up subjects reported by Duncan[12] and by Sanders[9] confirm our results but again the numbers are small. We have not recommended the cortisone-glucose tolerance test for routine diagnostic use as has been implied by others.[11]

It would seem appropriate, finally, to repeat our modest claims for the cortisone-glucose tolerance test with emphasis on some of the important words.

"The cortisone-glucose tolerance test has been devised and is being used as a *technical instrument of detection.* When used in a *standard way* it seems to separate the nondiabetic relatives of diabetics into groups which are distinctly different from the groups which are found when the same test is applied to people with no known family history of diabetes. Only *long-term future observations* on these various groups will tell us whether or not *this test,* or *some modification* of it, will be capable of detecting at an *earlier age* the great masses of people who are to make up our future diabetic populations. *When* we can detect the prediabetic *with reasonable certainty,* only then can we justify the use of therapeutic or prophylactic measures designed to prevent the disease or, at least, to prevent the 'decreased-insulin-activity-aspect' of the disease, that aspect about which we know the most. We believe that the present study represents a move, *minute* as it may be, in the right direction."[5]

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BOOK REVIEWS

ANNUAL REVIEW OF BIOCHEMISTRY. *Edited by J. Murray Luck. $7.00, pp. 783, Annual Reviews, Inc., Palo Alto, California, August 1960.*

This twenty-ninth volume of the series published annually since 1932 contains twenty-six topics in biochemistry. It is not meant to be an inclusive coverage but rather a discussion of the most important work of the preceding year and not a historical development of the various subjects. This volume covers the literature of the year 1959. These reviews have been an excellent source for scouting the literature, for reference work and for seeking new ideas. Thus, they have become a welcome aid for those engaged in teaching and in research.

Five chapters that ought to interest students of diabetes have been selected for comment: "The Mechanism of Enzyme Action" by P. D. Boyer is the first such review of this subject. He calls attention to the intricate events that occur in enzymatic catalysis. Many aspects of enzyme action, some well understood and some not understood, are discussed. P. K. Stumpf has written the chapter on "Lipid Metabolism" in which developments in the areas of fatty acid synthesis and oxidation and proprionic acid metabolism are discussed. A. Beloff-Chain and F. Pocchiari have reviewed "Carbohydrate Metabolism." The transport of carbohydrates, tissue permeability and clinical studies related to carbohydrate metabolism have not been considered. They have discussed (1) polysaccharide and disaccharide synthesis with particular reference to the systems that involve the uridine cofactors, (2) metabolism and biosynthesis of hexoses and pentoses, (3) glycolysis with particular reference to the enzyme systems involved and to the Pasteur effect, (4) alternate pathways of carbohydrate metabolism with special reference to the pentose phosphate, glucuronic acid and Entner-Deudoroff pathways, (5) hormones involved in carbohydrate metabolism, with emphasis on insulin and diabetes, pituitary and adrenal hormones.

G. N. Cohen and F. Gros surveyed "Protein Biosynthesis." This is of interest to those concerned with carbohydrate metabolism because of recent data that deal with the relationship of insulin to protein synthesis from labeled amino acids. Finally, R. Acher has written well on the "Biochemistry of Protein Hormones." The technics that led to the determination of the complete chemical structure of insulin and the chemical structure and the organic synthesis of oxytocin and vasopressin are being rapidly applied to other protein hormones. The present knowledge of the relation of protein structure to biological activity is given. This area of biochemical research provides an interesting and fascinating study of agents that have a close relationship to carbohydrate metabolism.

The good quality and reference value of all the reviews is again appreciated.

DIABETES (WITH A CHAPTER ON HYPOGLYCEMIA). *By 54 authors; edited by Robert H. Williams, M.D. $20.00, pp. 793, Paul B. Hoeber, Inc., New York, New York, 1960.*

The preface begins, "The recent tremendous progress in many facets of research dealing with metabolic changes in diabetes and its concomitants (complications) prompted the writing of the book for scientists with varied interests, but particularly for internists, basic medical scientists and students. It is a unique condensation of the latest and most authoritative information at both the basic science and clinical levels written in a clear and straightforward manner by more than forty experts from widely dispersed areas of the world. The authors were selected not only on the basis of their outstanding research contributions but also for their demonstrated effectiveness as teachers and authors. The editor made special efforts to attain coordination of the discussion throughout the book."

The forty-eight chapters which follow amply demonstrate the accuracy of the editor's introductory remarks; the book is a precise and concise presentation of the accumulated knowledge concerning the science and clinical aspects of diabetes. What it lacks in integration or suffers from an occasionally poor chapter is more than compensated for by the wealth of information contained within its covers; nineteen pages of indexed material and 1,141 selected references give some idea of the scope of the material covered. All students of medicine and the medical sciences will find this an excellent reference; those particularly interested in diabetes or in metabolic diseases will find it invaluable.

DIABETIC MANUAL. *By Elliott P. Joslin, M.D., Sc.D. $3.75, tenth edition, pp. 304, Lea & Febiger, Philadelphia, 1959.*

It is a very rare occurrence for the individual who has diabetes to be able to read the advice of a man who has treated great numbers of diabetics for sixty years. This manual lives up to its reputation gained through forty years or more of publication. Throughout the book the author's conviction is frequently reiterated, namely, that if the individual with diabetes follows treatment wisely, he will live long and happily. And, he says, early treatment exactly followed pays great dividends in the later years. Dr. Joslin admonishes the individual with diabetes to control his disease. By control, he means keeping the urine free of sugar, exercising regularly, and taking insulin or oral agents when they are required. Illustrating this point, the histories of some very unusual cases of diabetes who lived longer than the average nondiabetic are cited.

The cause of retinitis proliferans is due to lack of good control, Dr. Joslin asserts.

He believes in the use of oral hypoglycemic agents, and he himself prescribes tolbutamide. He says: "The discovery of these chemicals has resulted in an added respect for diet, exercise and the reduction of excess weight in the treatment of diabetes." He believes the use of the oral drugs will result in greater emphasis on diabetes and ultimately in improved treatment.

The young diabetic will be interested to read of Dr. Joslin's opinion regarding marriage. He says: "If marriage is contemplated by a diabetic, his or her future partner in both families should know the facts. Diabetes can be controlled, and diabetics can marry, live happily for years and years, and have healthy, nondiabetic children, but this is only a hundred per cent true when the diabetes is controlled, and there is diabetes in only one of the contracting parties."

Another statement from Dr. Joslin which shows his intense optimism: "There is never a greater opportunity for the discovery of new methods of treatment of diabetes than today, and he is certainly pessimistic who would forbid the marriage of diabetics upon the ground that the advance in the therapy of diabetes has reached an impasse."

About the presence of obesity in the diabetic, he says: "Nine out of ten diabetics whose disease began after thirty years of age were overweight at some period of their lives." And, again, he says, "I fear I am a little hard-hearted toward my obese friends," meaning that he believes reduction diets should be constructed on a radical reduction in the amount of fat.

From the dietary standpoint, some of the data on the carbohydrate value of foods can be challenged on the grounds that Dr. Joslin classifies the green vegetables still as 3 per cent, in spite of the fact that the value of available carbohydrate is well known to be closer to 1 per cent, or even lower in some instances. Again he teaches his patients the per cent of carbohydrate in various classes, whereas the great majority of dietitians all over the country prefer teaching the value of an average helping.

Every diabetic can profit by reading this manual because it will teach him to live successfully with diabetes.

HOW TO LIVE WITH DIABETES. *By Henry Dolger, M.D., and Bernard Seeman. $3.95, pp. 192, W. W. Norton & Company, Inc., New York, New York, 1959.*

This book is well written and should be of interest to patients and physicians alike. The chapters dealing with the history of our knowledge and the background of the disease are readable and accurate. The reader is given an unbiased presentation of the problems concerning management and the facilities available to the physician and all ancillary personnel concerned with the treatment of diabetics. The authors show an excellent understanding of the psychology and emotional problems confronting the juvenile diabetic, and the section on special problems for women is interesting and instructive. The authors gaze into the crystal ball in forecasting future developments in the management of this disease and here there may be some individuals who will question their predictions, but in general the book is factual and extremely readable.

However, several unfortunate facts detract from the book. It seems to this reviewer that there is undue emphasis on the efficacy of oral agents in general and one in particular. The matter of primary and secondary failures of response to oral agents is insufficiently discussed. It also would be preferable if the chemical or generic, rather than the proprietary, name for the compound were used.

THE CHEMISTRY OF HEREDITY. *By Stephen Zamenhof, Ph.D. $4.25, pp. 106, Charles C Thomas, Springfield, Illinois, 1959.*

Investigations over the past few years have defined in biochemical terms many features of the apparatus of heredity. Recent knowledge of the structure of nucleic acids, the enzymology of their synthesis, the sequence of biochemical events in the multiplication of viruses and organisms, and the definition of some phenotypes in terms of the amino acid sequence of a protein, are the chief foundation of the area that Stephen Zamenhof has described in his short monograph as "The Chemistry of Heredity." The fascination of this field is that it brings us to one of the fundamental problems of biology with a knowledge of certain parts of the heredity mechanism, but with many relationships still obscure and the gap between the chemical and the genetic approach to the gene as yet unclosed. The variety of speculations which has been suggested to fill in these uncertainties has been scientifically stimulating, but has left many not actively working in this field unable to distinguish fact from fancy.

It is the great virtue of Dr. Zamenhof's book that the biochemical aspects of the problem of heredity are clearly set forth with a real communication of the nature of the evidence and its degree of relevance to the problem. Since this is done without assuming any chemical background or prior acquaintance with the material on the part of the reader, the book provides an excellent account, complete in itself, for one with some elementary knowledge of biology. At the same time, because the bibliography is unusually extensive, the more serious student can obtain a perspective and the means for further study.

The early chapters on heredity determinants as chemical substances and on the nucleic acids are especially successful. The Watson-Crick double helix and its catalytic impact on ideas relating nucleic acid structure to biological function are considered in detail. Of the later chapters, the one on hereditary defects in man is too brief to present the main ideas adequately. The book as a whole is another demonstration that the best popularizer of a scientific field is a scientist who has made contributions to it and who has taken the trouble to explain them.

THE CHEMISTRY OF LIPIDS IN HEALTH AND DISEASE. *By H. K. King, M.A., Ph.D., F.R.I.C. $3.75, pp. 104, Charles C Thomas, Springfield, Illinois, 1960.*

This small monograph is a well-organized presentation of the chemistry and metabolism of lipids. An introductory chapter, "Lipids and Water," describes the factors of molecular structure and orientation that determine the behavior of lipids in an aqueous medium. The chemical structures of various types of lipids are considered in Chapter 2, the digestion and absorption of fats are reviewed historically, and the present status is described in Chapter 3. Unfortunately, the absorption and metabolism of sterols receives inadequate treatment in a very short section. The succeeding two chapters are devoted to the oxidative breakdown of fats and the biosynthesis of lipids.

The final two chapters, on lipids in disease, are skimpy and do not fulfill the promise of the title of the monograph. Chapter 7 contains material on dietary essential fatty acids and fat-soluble vitamins that may be found in texts on biochemistry, and much speculative material is offered in the final chapter on lipids and atherosclerosis. No mention is made of the chemistry or metabolism of lipids in such other well-known entities as Schüller-Christian's disease, Gaucher's disease or other lipidoses, biliary cirrhosis, the various types of steatorrhea, or, of particular interest to the readers of this journal, diabetes.

Despite these shortcomings, the monograph is extremely readable, and the basic aspects of the chemistry and metabolism of lipids are presented with considerable clarity.

ABSTRACTS

Alterman, Seymour L. (Mount Sinai Hosp., Miami Beach, Fla.): DIABETES, ATHEROSCLEROSIS AND HYPERCHOLESTEREMIA: A PRELIMINARY REPORT ON THE STUDY OF DIETARY CONTROL. Postgrad. Med. *28*:112-20, August 1960.

The author reviewed the evidence regarding the relationship between the acceleration of atherosclerosis in diabetes and the blood cholesterol levels in diabetes. He also reviewed the evidence for reduction in such levels by reduction of saturated and substitution of unsaturated fats in the diabetic diet. He discussed the construction of such a diet, modified largely by the use of lean protein and margarine made from unsaturated corn oil. S.B.B.

Barta, L. (University of Budapest, Hungary): FAMILIAL INCIDENCE OF DIABETES MELLITUS AMONG DIABETIC CHILDREN IN HUNGARY. German M. Month. 5:228-29, July 1960.

The familial incidence of diabetes mellitus was found to be 15.4 per cent among 227 diabetic children, 5.5 per cent among 251 obese but not diabetic children, and 2 per cent among 900 healthy children. The parental incidence of diabetes in the three groups was 2.7 per cent, 28 per cent and none, respectively. While socio-economic factors were of importance in determining the manifestation of diabetes in adults, this factor played little role in children. W.R.K.

Beckett, A. Gordon; and Lewis, J. G. (Royal Free Hosp., London, W.C.1, England): MOBILIZATION AND UTILIZATION OF BODY-FAT AS AN AETIOLOGICAL FACTOR IN OCCLUSIVE VASCULAR DISEASE IN DIABETES MELLITUS. Lancet *2*:14-18, July 2, 1960.

An association between the onset of serious vascular disease and the period of planned therapeutic weight loss is reported. The authors speculate that increased rate of fat metabolism during weight loss and diabetes may produce a relative deficiency of essential unsaturated fats precipitating thereby a disequilibrium of blood fats and coagulation to produce thrombosis or acceleration of atherogenesis. J.A.

Bertrand, N. R.; and Gilbert, J. A. L. (Dept. of Obstet. & Med., Univ. of Alberta Hosp., Edmonton, Alberta, Canada): THE PREDNISONE GLUCOSE TOLERANCE TEST IN THE DIAGNOSIS OF DIABETES IN WOMEN PRODUCING LARGE BABIES. Canad. M.A.J. *83*:753-55, Oct. 1, 1960.

The carbohydrate tolerance was studied among sixty women who had been delivered of a baby of 9 lb. or over six months to five years previously. Both a standard glucose tolerance and one after 25 mg. of prednisone were performed. A parallel group of forty-five women who had been delivered of average-sized babies served as controls.

Only two of the sixty women had a positive response with the standard glucose tolerance and 50 per cent with the steroid glucose tolerance test. There was no positive response with the former among the controls, but a 31 per cent response with the latter test. Factors such as age, obesity, parity, percentage incidence of diabetic relatives or perinatal fetal losses were essentially the same among the positive and the negative responders. The significance of these findings and of the steroid stress test was discussed. S.B.B.

Blumenthal, Herman T.; Alex, Morris; and Goldenberg, Sidney (St. Louis Univ., Sch. of Med., Washington Univ. Sch. of Med., St. Louis, Mo.): A STUDY OF LESIONS OF THE INTRAMURAL CORONARY ARTERY BRANCHES IN DIABETES MELLITUS. Arch. Path. 70:13-28, July 1960.

Lesions of the intramural coronary artery branches were studied and tabulated with respect to age, sex, diabetes mellitus, and hypertension. Autopsy evidence included 116 diabetic and 105 nondiabetic cases.

Fibrous intimal thickening, atheromata with or without embolization and thrombosis, and inflammatory arteritis were encountered in approximately equal numbers in both groups. However, endothelial proliferative lesions of PAS positive materia in intramural branches were found 2.5 times more often in the diabetic cases.

The greater frequency of myocardial infarction, recurrent infarction, myocardial failure, and myocardial rupture in diabetic individuals suggests an impaired potential for developing adequate collateral circulation. The intramural site of the proliferative lesion may account for this impairment. H.L.W.

Boley, Scott J.; Lin, Jane; and Schiffmann, Albert (Dept. of Pediatrics, Jewish Hosp., Brooklyn, N.Y.): FUNCTIONING PANCREATIC ADENOMAS IN INFANTS AND CHILDREN. Surgery *48*:592-605, September 1960.

The world literature on functioning islet cell adenomas of the pancreas in children under the age of fifteen years has been reviewed and a new case added. The clinical, laboratory, operative, and autopsy findings in these cases have been studied in an attempt to establish criteria to differentiate functioning insulinoma from idiopathic spontaneous hypoglycemia of infancy and childhood. The age at the onset of symptoms is the most important diagnostic criterion. No gross tumor was reported in patients under four years of age. No familial tendency was reported nor was there any sex preponderance. Conservative therapy is recommended for children under four years of age with operation reserved for those patients whose disease is not controlled with ACTH. Surgical exploration is advised for all children under four years of age after an evaluation of response to ACTH has been made. In this age group blind pancreatectomy is indicated if no gross tumor is found and the preoperative response to ACTH was unsatisfactory. W.R.K.

Bolinger, Robert E.; Tu, Wu-Hao; and Kendall, Charles (Dept. of Med., Univ. of Kansas School of Med., Kansas City, Kans.): ORAL HYPOGLYCEMIC AGENTS: CLINICAL EXPERIENCE WITH FIFTY DIABETIC PATIENTS. J. Kansas M. Soc. 61:135-41, March 1960.

Experiences with management of fifty diabetic patients with oral hypoglycemic agents are reported. A good response was obtained in about half of the patients treated with the sulfonylureas and failure occurred in about one fifth of the cases tried. The incidence of successes increased after age forty-five and in patients taking less than 20 units of insulin. Several incidences of refractoriness to one of the sulfa drugs and responsiveness to another were noted. DBI had the additional ad-

vantage over the sulfonylureas in being effective in combination with insulin in young, "brittle" diabetics. Reactions to be guarded against with the sulfonylureas drugs are hypoglycemia and ketosis in refractory patients. W.R.K.

Bounous, G.; and Shumacker, H. B., Jr. (Dept. of Surgery, Indiana Univ. School of Med,, Indianapolis, Ind.): HEMODYNAMIC EFFECTS OF INTRAVENOUS INJECTIONS AFFECTING THE LEVEL OF BLOOD SUGAR. Ann. Surg. *151*:453-59, April 1960.

Rapid intravenous injection of 50 per cent dextrose (approximately 25 gm.) into anesthetized dogs produced a temporary twofold rise in cardiac input, a 40 per cent rise in blood volume and moderate fall in blood pressure. These changes disappeared within five minutes. They could be duplicated by infusion of 250 cc. of whole blood but there was no change following insulin injection. Glucagon administration (dose, 3 mg.) caused minimal blood volume and pressure alterations but the increase in cardiac input was lacking. There was no correlation between renal blood flow and cardiac output or blood volume. It is felt that hypertonic dextrose solution lowers the vascular resistance by decreasing arteriolar sphincter tonus. A.R.C., JR.

Bounous, G.; and Shumacker, H. B., Jr. (Dept. of Surgery, Indiana Univ. Sch. of Med., Indianapolis, Ind.): INFLUENCE OF BLOOD SUGAR LEVELS UPON RENAL BLOOD FLOW. Ann. Surg. *151*:441-52, April 1960.

Intravenous injection of 5, 10 or 50 per cent dextrose in water over one-half hour produced an immediate rise in renal blood flow in anesthetized dogs, which corresponded to the amount of dextrose introduced. Dosage amounted to approximately 1 or 2 gm. of dextrose per kilogram of body weight. Glucagon administration (1 to 3 mg.) caused a similar change. Renal venous oxygen saturation and urinary volume showed increases which varied directly with the renal blood flow. Injection of saline or whole blood failed to bring about these effects. Administration of insulin (0.5 U./kg.) intravenously reduced renal blood flow to 60 per cent of control values within one-half hour. Blood glucose level correlated well with renal blood flow and urinary volume. Since a fall in blood glucose stimulates adrenalin secretion, the reduction in blood flow may be due to renal vasoconstriction. Hyperglycemia may inhibit adrenal medullary secretion. A.R.C., JR.

Brandt, Ralph L. (Medical School, Univ. of Mich., Ann Arbor, Mich.): DECREASED CARBOHYDRATE TOLERANCE IN ELDERLY PATIENTS. Geriatrics *15*:315-25, April 1960.

A very high incidence of decreased carbohydrate tolerance has been found in a select aged population using criteria which have been established for a younger active healthy population. Abnormal carbohydrate metabolism was found to correlate most strongly with age. There was a lack of correlation between decreased carbohydrate tolerance and eating habits, obesity and sex. The cortisone-glucose tolerance test was utilized for the study of carbohydrate metabolism in this study. A significant number of individuals had positive responses and this was especially true of those with borderline, baseline glucose tolerance tests. From this study it is uncertain if decreased carbohydrate tolerance in the aged is synonymous with the diagnosis of diabetes mellitus as applied to younger subjects. Because of the high prevalence of the diminished carbohydrate tolerance in the older age group, it is felt that the diagnosis of diabetes mellitus in these patients should be made with caution. S.S.

Bühring, H.; and Kühnau, J. (Dept. of Physiol. Chem., University Hamburg, Germany): THE TOTAL AND THE ACETYL CO-ENZYME A CONTENT OF THE LIVERS OF NORMAL AND ALLOXAN-DIABETIC RATS. Klin. Wchnschr. *38*:694-98, July 15, 1960.

Co A was estimated using the method of Kaplan and Lipmann, the acetyl-Co A using a method developed by Bühring. The values were expressed in LU/gm. (Lipmann units/gram). It was found that both the Co A and the acetyl Co A contents of the liver undergo marked seasonal fluctuations in the normal as well as in the alloxan diabetic rat. At all times, however, the alloxan diabetic liver had a markedly higher content than the normal liver. In the normal liver the Co A varied between 82 and 160 LU/gm. and the acetyl Co A between 4 and 17 LU/gm., the respective ranges for the alloxanized liver were 113-355 LU/gm. and 7-35 LU/gm. The highest values occurred during the summer months, the lowest during the winter. The seasonal changes are considered to be due to hormonal influences, since the animals had been kept under constant laboratory conditions and had been sacrificed after a forty-eight-hour period of starvation. The elevation of Co A and acetyl Co A in the diabetic liver is interpreted as a significant metabolic disturbance in lipogenesis which may be the primary cause for the overproduction of cholesterin and of acetone bodies in the diabetic organism (German). M.G.G.

Butterfield, W. J. H.; Fry, I. Kelsey; and Whichelow, M. J. (London, S.E.1, England): SOME OBSERVATIONS ON THE EFFECT OF SMALL DOSES OF GLUCAGON IN NORMAL AND DIABETIC SUBJECTS. Guy's Hosp. Rep. *109*:95-109, 1960.

The use of glucagon infusions in the study of carbohydrate metabolism is discussed. The importance of using the smallest effective dose of glucagon is emphasized and a test is described in which 2γ per minute are infused for twenty minutes. The results obtained using this test in twenty-five diabetic patients have been compared with those in twelve control subjects. The response in the diabetics was smaller and started more slowly. The effect of successful treatment with tolbutamide on the response to glucagon has been studied in four patients. After tolbutamide the response was greater and began earlier. The uptake of glucose by the forearm tissues after glucagon has been compared with that after glucose given to produce a comparable degree of hyperglycemia in three patients. Glucagon was found to have no effect on peripheral glucose uptake. The use of the glucagon test as a guide to the availability of liver glycogen is discussed. The improved response to glucagon in diabetes after successful treatment with tolbutamide is considered in the light of current studies of the mode of action of this drug. W.R.K.

Chalmers, T. M.; Pawan, G. L. S.; and Kekwick, A. (Dept. of Med. Middlesex Hosp., Med. Sch., London W.1, England): FAT-MOBILIZING AND KETOGENIC ACTIVITY OF URINE EXTRACTS: RELATION TO CORTICOTROPHIN AND GROWTH HORMONE. Lancet *2*:6-9, July 2, 1960.

A polypeptide-like substance has been isolated from the urine of people actively mobilizing and utilizing fat. This material gives rise to transient hypoglycemia, ketonemia, and

increased mobilization and catabolism of fat in mice with depletion of body fat stores. In vitro experiments show that at a concentration of 1 μg./ml. this substance causes the release of free fatty acids from rat adipose tissue. The pituitary was necessary for the production of the compound and it is apparently not growth hormone or ACTH. J.A.

Cochrane, W. A. (Dept. of Pediat. Faculty of Med., Dalhousie Univ., Halifax, N.S., Canada): STUDIES IN THE RELATIONSHIP OF AMINO ACIDS TO INFANTILE HYPOGLYCEMIA. A.M.A. J. Dis. Child. 99:476-88, April 1960.

The subject has been investigated extensively from several aspects. Twelve children with idiopathic infantile hypoglycemia were given casein hydrolysate by mouth and six of these had a significant drop in blood sugar. These six were tested with *l*-leucine and several other amino acids. They all showed marked sensitivity to *l*-leucine as manifested by a drop in blood sugar. Other amino acids tested had no effect except for a moderate response to *d*-leucine in two infants. Intravenous administration of *l*-leucine produced a significant drop in blood sugar in normal rats but not in rabbits. Results in dogs were inconclusive. The uptake of glucose by isolated rat diaphragm was increased by the addition of *l*-leucine to the incubation media, but not by addition of tryptophane. Studies on the release of glucose by rat liver slices suggests that *l*-leucine may inhibit glycogenolysis.

The author discusses the possible implications of this and other work with regard to the mechanism of action of *l*-leucine on blood sugar.

A plan of treatment of idiopathic infantile hypoglycemia is presented. This includes the use of cortisone or corticotropin combined with extra carbohydrate thirty minutes after feeding. A low leucine diet might be considered for severely affected infants who are found to be markedly sensitive to leucine. R.L.J.

Dampeer, T. K., Jr. (Dept. of Obstet. & Gynec., Tulane Univ. School of Med., New Orleans, La.): DIABETES AND PREGNANCY. J. Mississippi M. A. 1:351-55, July 1960.

The experiences at the New Orleans Charity Hospital in the management of eighty-eight consecutive cases of diabetes in pregnancy are presented. The perinatal mortality was 33 per cent with no maternal deaths. The complications and altered physiology throughout pregnancy and the puerperium have been discussed. It is stressed that best results are obtained by an awareness of the possibility of deranged carbohydrate metabolism coupled with thorough medical management throughout pregnancy and delivery. W.R.K.

Dillingham, C. H. (Albuquerque, N.M.): FAMILIAL OCCURRENCE OF HEMOCHROMATOSIS. New England J. Med. 262: 1128-30, June 2, 1960.

Four cases of hemochromatosis in siblings of one family of Spanish-American ancestry are reported. The first patient, a forty-year-old woman, was diabetic for two years. The second case, a sister, had been diabetic for eighteen years. The third case, a brother thirty-five years of age, was diabetic for five years and a thirty-year-old sister was not aware of diabetes but on examination was found diabetic. It is suggested that relatives of patients with hemochromatosis be screened more carefully in the hope of detecting more cases at early dates. S.S.

Dodds, Sir Charles; Miller, A. L.; and Rose, C. F. M. (Courtauld Inst. of Biochem., Middlesex Hosp., London W.1, England): BLOOD PYRUVATE AND LACTATE RESPONSE OF NORMAL SUBJECTS TO DEXTROSE, SUCROSE, AND LIQUID GLUCOSE. Lancet 2:178-80, July 23, 1960.

Pyruvate and lactate levels showed a greater increase following sucrose than either dextrose or liquid glucose. The higher levels of metabolites seen after sucrose may, the authors suggest, come from hepatic metabolism of the fructose moiety of sucrose. J.A.

Dosekun, F. O.; Grayson, J.; and Mendel, D. (Dept .of Physiology, University College, Ibadan, Nigeria): THE MEASUREMENT OF METABOLIC AND VASCULAR RESPONSES IN LIVER AND MUSCLE WITH OBSERVATIONS ON THEIR RESPONSES TO INSULIN AND GLUCOSE. J. Physiol. 150:581-606, March 1960.

Measures of heat production were taken and blood flow in the liver and (gastrocnemius) muscle of Wistar rats using a heated thermocouple method. The resting heat production in rat liver was about ninety-five times that in resting muscle, per gram of tissue. Intravenous insulin caused a 44 per cent increase in liver heat production and a 670 per cent increase in muscle heat production. Intravenous glucose depressed both liver and muscle heat production. Combinations of intravenous insulin *plus* glucose produced variable but usually slight effects in the liver, but greatly increased heat production in muscle. The possible relationship of these effects to glycogen breakdown is discussed. Insulin usually produced vasodilatation in the liver and, possibly, in muscle, which could be prevented by the simultaneous administration of glucose. G.A.W.

Editorial (National Institutes of Health, Bethesda, Md.): DIABETES. Highlights of Research 1959: Progress in Arthritis and Metabolic Diseases. Public Health Service Publication No. 753, page 9, 1960.

Diabetes is probably the best known and most important of the metabolic diseases. It results from either an insufficient production of insulin by the pancreas, or from interference with insulin's action after it has been produced. Because of this abnormality, the diabetic patient is unable properly to utilize sugar (glucose) and excess amounts of it build up in the blood and spill over into the urine. It is an extremely complex disorder which is now known to encompass alterations in fat and protein metabolism as well as sugar metabolism. There is still much to be learned about the basic mechanisms that are operating in this common metabolic disease. More research on the spatial configuration and function of insulin is needed, and because of many interrelationships, the biochemistry and metabolism of other regulators—especially the hormones of the pituitary and adrenal glands—must be further investigated. The recent widespread use of the new oral antidiabetic drugs has brought about major changes in the treatment of thousands of diabetics who have, under medical supervision, exchanged their regular insulin injections for one of the new tablets. Their ultimate value in the treatment of diabetes remains to be seen, but at the very least they have played a valuable part in fostering a dramatic new surge of scientific interest in this age-old disease. W.R.K.

Engelhardt, Hugo T.; and Snyder, Harvey B. (Dept. of Medicine, Baylor Univ. College of Medicine, Houston, Tex.): THE DIABETIC IS EMPLOYABLE. J. Occupational Med. 2:427-31, September 1960 (also reprinted in M. Bull. 20:397-405, November 1960).

The diabetic adult who requires no insulin to control his

disease presents no problem to management. For purposes of employment, persons in this category may be considered in many instances in the same fashion as nondiabetics. At the other extreme is the uncooperative and poorly regulated diabetic job applicant whom every employer is justified in refusing employment. Such cases can usually be effectively screened out by pre-employment interview and examination procedures. Between these two extremes, there is a large group of diabetics who follow their physician's advice and understand their disease and, as a result, are well controlled. When these persons are given appropriate positions, they make good employees. W.R.K.

Fineberg, S. K. (Medical Service, Harlem Hosp., New York, N.Y.): CLINICAL EXPERIENCE WITH CHLORPROPAMIDE AND COMPARATIVE EVALUATION WITH TOLBUTAMIDE. J. Am. Geriatrics Soc. *8*:441-48, June 1960.

A clinical study of the effect of chlorpropamide in fifty patients with "maturity-onset" diabetes is presented, and compared with the effect of tolbutamide in a similar but smaller group of thirty-five patients selected by the same criteria. The findings of these studies were: (1) chlorpropamide produced satisfactory control of the diabetes in almost twice as great a percentage (76 versus 43 per cent) of patients than did tolbutamide, and excellent control in more than twice as great a percentage (74 versus 31 per cent); (2) the incidence of primary and secondary failure with tolbutamide therapy was notably higher than with chlorpropamide therapy; and (3) with the dosage levels used, chlorpropamide produced as few, and possibly fewer, side reactions than did tolbutamide. Further comparative evaluations would be of value in the final assessment of these two clinically effective oral hypoglycemic agents. W.R.K.

Frank, Stanley T.; Clark, George C.; and Hill, S. Richardson (Univ. Hosp. and Hillman Clinic, the Univ. of Alabama Med. Center, Birmingham, Ala.): PODIATRY AND DIABETES MELLITUS. J. M. A. Alabama *29*:434-35, May 1960.

After a period of careful observation and evaluation it is possible to report that the establishment and operation of a Podiatry Clinic has aided greatly the mission of the Diabetes Clinic in the care of the diabetic patient. It should be emphasized, of course, that the treatment and control of the diabetes supersedes any local foot treatment. If the patient's blood sugar is then within normal limits, treatment and cure of foot problems are greatly facilitated. Finally, it is important to maintain close liaison at all times with the Surgical Clinic for Peripheral Vascular Diseases for more complicated problems. It can be concluded, however, that consideration should be given to the inclusion of podiatrists in the care of the diabetic patient to work under the direction of the physician in the prevention and correction of foot problems of nonsurgical nature. W.R.K.

Friedlander, Ernest O. (Med. Serv., Veterans Administration Center, Togus, Me.): USE OF ACTH AND ADRENOCORTICAL STEROIDS IN IDIOPATHIC INSULIN-RESISTANT DIABETES. J. Maine M. A. *51*:229-32, July 1960.

A diabetic patient with severe recurrent idiopathic insulin resistance is described who has been observed over a six-year period. During this time use of ACTH and adrenocortical steroids on three occasions resulted in a reduction of the daily insulin requirement to 19 per cent, 16 per cent, and 17 per cent of the respective initial dose. The management of idiopathic insulin resistance with special reference to the use of adrenocortical steroids is outlined. The salutary effect of the adrenocortical hormones is attributed to the suppression of insulin antibody formation. Attention is directed to the fact that the response to treatment often is slow and gradual and that occasionally an initial increase of the daily insulin dose may be expected. A prolonged administration of the steroids is necessary to prevent treatment failure. W.R.K.

Gabric, Dora; and Allegretti, Niksa (Medical Faculty, Univ. of Zagreb, Yugoslavia): SOME CHANGES OF APPETITE IN ALLOXAN DIABETIC RATS. Endocrinology *67*:285-87, August 1960.

Adaptation in appetite to the requirements of the animal has been described. Alloxan diabetic rats and normal rats were allowed to choose any of several drinking solutions. The alloxanized animals showed a preference for glucose solution, tap water, and Na_2HPO_4, while the normal animals preferred solutions of NaH_2PO_4 and NaCl. It is suggested that the preference for glucose by the diabetic rat may be related to its relative glucose deprivation due to glycosuria. Consumption of Na_2PO_4 may be related to the diminished alkali reserve in the diabetic rat. H.L.W.

Godlowski, Z. Z.; and Calandra, J. C. (Dept. of Pathology and Med. Diagnosis, Dental Sch., Northwestern University, Chicago, Ill.): HYPOGLYCEMIC EFFECT OF TOLBUTAMIDE MEDIATED BY ARGENTAFFINE CELLS. J. Appl. Physiol. *15*: 684-86, July 1960.

This reports a study showing that in the absence of both the submaxillary glands and the complete intestinal tract, the hypoglycemic effect of tolbutamide could not be demonstrated. They suggest that the hypoglycemic action of the drug is mediated by the argentaffine cells. J.A.

Gofman, John W. (Univ. of Calif., Berkeley, Calif.): DIETARY CARBOHYDRATE AND BLOOD LIPID LEVELS. Illinois M. J. *118*: 82-83, August 1960.

Patients with coronary artery disease tend to show higher levels of blood lipids than matched healthy individuals. Which blood lipids are most important remains to be determined although both cholesterol and Sf 0-20 lipoproteins on the one hand, and triglycerides and Sf 20-400 lipoproteins on the other have been associated with coronary disease.

Consumption of diets high in animal or saturated fat tends to increase Sf 0-20 lipoproteins rich in cholesterol. Intake of a high CHO diet tends to increase the Sf 20-400 triglyceride rich fraction. The physician prescribing diets for patients with coronary disease must weigh carefully the effects of his therapy. H.L.W.

Goto, Yoshio; Kato, Joji; Takanami, Akira; and Ohneda, Akira (Medical Dept., Tohoku Univ. Medical Faculty, Sendai, Japan): DETECTION OF PREDIABETES BY GLUCOSE TOLERANCE TEST SENSITIZED BY PREDNISOLONE. Lancet *2*:461-65, Aug. 27, 1960.

The average response index, which is the sum of the blood sugar differences between the standard curve and the prednisolone-sensitized curve at each time point, and which represents the intensity of the reaction to prednisolone, was fifteen for normal subjects, fifty for the nondiabetic patients, 110 for blood relatives of diabetics, 124 for patients with pancreatic

disease and 189 for the diabetic patients. Details as to age, clinical condition, etc., of the above classifications, are given. J.A.

Grodsky, Gerold M.; and Forsham, Peter H. (Metabolic Unit for Research in Arthritis and Allied Diseases and the Dept. of Medicine, University of Calif. Sch. of Med., San Francisco, Calif.): AN IMMUNOCHEMICAL ASSAY OF TOTAL EXTRACTABLE INSULIN IN MAN. J. Clin. Invest. 39:1070-79, July 1960.

A new method of immunological assay of insulin is described. Its specific application to human serum was made more precise by utilizing acid-alcohol for extraction of the serum and 15 per cent urea to remove extraneous proteins in the extracts. Guinea pig antibovine insulin antibody was used for assay.

The minimum sensitivity of the assay was 20 micro-units per ml. of serum and there was less than this amount in normal human serum. A rise occurred to a mean level of 31 micro-units thirty minutes after oral glucose with a marked decline in sixty minutes. The serum of five patients with acromegaly was assayed and an increase found in four. In insulin resistant diabetes an increased level (3,100 micro-units per ml.) was found twenty-four hours after the injection of 500 units of insulin at a time when the diabetes was still uncontrolled (blood sugar 300 mg. per 100 ml.). S.B.B.

Hagen, Jean Himms; and Ball, Eric G. (Dept. of Biol. Chem., Harvard Med. Sch., Boston, Mass.): STUDIES ON THE METABOLISM OF ADIPOSE TISSUE. IV. THE EFFECT OF INSULIN AND ADRENALINE ON GLUCOSE UTILIZATION, LACTATE PRODUCTION, AND NET GAS EXCHANGE. J. Biol. Chem. 235:1545-49, June 1960.

Glucose consumption, lactate production and net gas exchange were measured on the rat epididymal fat pad in a bicarbonate medium. Addition of adrenalin caused a delay in gas exchange, an increase in lactic acid production and a reduction of the total carbon dioxide output induced by insulin. The findings suggest that adrenalin interferes with the conversion of glucose to fatty acid with diversion of glucose metabolism to other metabolic products. A.R.C., JR.

Haining, R. B.; and Haining, R. G. (540 North Central Ave., Glendale, Calif., and Boston, Mass.): SPONTANEOUS REMISSION OF DIABETES. California Med. 92:436-39, June 1960.

A sixty-two-year-old woman was hospitalized in February 1954 with hyperglycemia and mild acidosis. She was given insulin and intravenous fluids and a restricted diet was prescribed. Within four hours the laboratory signs of diabetic acidosis were reversed. Insulin dosage was gradually reduced and in four weeks was discontinued. She adhered to a mildly restricted diet till October 1954. Since then she has not modified her food intake in any way except to reduce consumption of cholesterol-rich foods. Glucose tolerance tests indicate that she is still a latent diabetic. Nevertheless, in the past six years a number of severe infections, some requiring admittance to hospital, have not provoked signs or symptoms of diabetes. W.R.K.

Halprin, Harry (145 Union St., Montclair, N.J.): HYPERGLYCEMIC REACTION TO A HYDROCHLOROTHIAZIDE. J. M. Soc. New Jersey 57:254-55, May 1960.

One of the modern hydrochlorothiazides, Esidrix (Ciba) is a potent oral diuretic. This is the report of a seventy-four-year-old nondiabetic who had had signs of multiple myocardial infarctions since 1947. He had signs and symptoms of chronic decompensation. In December 1958 he was given Esidrix as described below. At times his blood sugar reached 412 mg. per 100 ml. He was placed on a diabetic diet and given tolbutamide. The hydrochlorothiazide was discontinued and the blood sugar dropped. Later he returned to the hospital and the Esidrix was given again. And once again his blood sugar went up—this time to 185 mg. per 100 ml. At this level the hydrochlorothiazide was discontinued and chlorothiazide was administered. The chlorothiazide did not have the hyperglycemic effect. Certain special circumstances in this elderly man could have disturbed the normal carbohydrate metabolism. His repeated and persistent bouts of myocardial failure, liver congestion caused by myocardial decompensation, or some enzyme insufficiency as well as the hypochloremic alkalosis—all, or any of these factors may have been primarily responsible for the disturbed metabolism. Yet the finger of suspicion seems to point to the hydrochlorothiazide. W.R.K.

Hargrove, Marion D., Jr.; Verner, John V.; and Ruffin, Julian M. (Duke Univ. Med. Center, Durham, N.C.): DIABETES AND CARCINOMA OF THE PANCREAS. South. M. J. 53:706-08, June 1960.

The incidence of carcinoma of the pancreas in diabetics has long been known to be higher than in the population at large. This lesion should be suspected if weight loss appears in spite of satisfactory regulation of the diabetes. Diabetes developing in a middle-aged person who has no family history of diabetes should raise the diagnosis of pancreatic cancer for consideration. Three patients are reported, illustrating different aspects of disturbed carbohydrate metabolism in association with carcinoma of the pancreas. The development of an abnormal glucose tolerance test in the thin middle-aged or elderly individual should make one suspicious of the presence of carcinoma of the pancreas. The progressive downhill course, in spite of adequate diabetic regulation in the longstanding diabetic, is strongly suggestive of carcinoma of the pancreas. W.R.K.

Harwood, Reed (Massachusetts Gen. Hosp., Boston, Mass.): INSULIN-BINDING ANTIBODIES AND "SPONTANEOUS" HYPOGLYCEMIA. New England J. Med. 262:978-79, May 12, 1960.

An unusual case of diabetes is described in which insulin therapy must be abandoned for a few days periodically because of prolonged hypoglycemia. Proof is offered that the patient does omit insulin as she claims. A partial explanation of this unusual phenomenon is found in tests of her serum globulins, which show an unusually high insulin-binding capacity and an unusually slow rate of dissociation of the insulin-antibody complex. W.R.K.

Henneman, Dorothy H.; and Henneman, Philip H. (Present address: Seton Hall College of Medicine, Jersey City, N.J.): EFFECTS OF HUMAN GROWTH HORMONE ON LEVELS OF BLOOD AND URINARY CARBOHYDRATE AND FAT METABOLITES IN MAN. J. Clin. Invest. 39:1239-45, August 1960.

Human growth hormone was administered to five patients with hypopituitarism and one with obesity. The dose was sufficient to cause prompt nitrogen retention and growth. The latter effects waned after two to three weeks of administration. There was an early and substantial rise in plasma free fatty acids and serum and urinary citrate. There was a decrease in

fasting blood glucose but an early and sustained decrease in glucose tolerance (blood sugar one and two hours after a meal). Unlike the nitrogen and growth effects, these did not wane. All effects disappeared after a twelve-day interval without growth hormone therapy. S.B.B.

Hobkirk, R.; Blahey, P. R.; Alfheim, A.; Raeside, J. I.; and Joron, G. E. (The Montreal Gen. Hosp., Montreal, Quebec, Canada): URINARY ESTROGEN EXCRETION IN NORMAL AND DIABETIC PREGNANCY. J. Clin. Endocrinol. 20:805-13, June 1960.

Urinary levels of estriol, estrone and estradiol-17*B* (estradiol) increase during the last twenty weeks of pregnancy in a qualitatively similar fashion in both the normal and diabetic states. The relative amounts of these estrogens found in the urine suggest that they arise from the same source in both groups according to the transformation, estradiol⇄estrone→estriol. It also appears likely that there is an additional source of estriol in the two groups, particularly in late pregnancy. There is a tendency for estriol levels to be lower in diabetic than in nondiabetic pregnancy. Considerable variability in the relative amounts of these three estrogens from one pregnancy to another, both diabetic and normal, suggests the occurrence of a varying pattern of estrogen metabolism from one individual to another. W.R.K.

Hümmer, N.; Lehrnbecher, M.; and Moeller, J. (Univ. of Würzburg and Municipal Hosp., Hildesheim, Germany): RENAL FUNCTION AND ORAL ANTIDIABETIC DRUGS. German M. Month. 5:257-61, August 1960.

Repeated examinations of renal functions over many months were conducted on one hundred diabetics, forty-three of whom required insulin, nineteen diet only, and thirty-eight either carbutamide or tolbutamide. Carbutamide and insulin administration led to a similar degree of improvement in renal functions. Patients with a glomerular filtration rate of below 100 ml./min. responded well to oral hypoglycemic medication; this may explain the failure of oral medication in juvenile diabetics, because these have a high glomerular filtration rate. In another series carbutamide clearances were measured in nineteen diabetic and eleven nondiabetic subjects with various levels of glomerular filtration. Here again, the response to oral medication was most marked in those patients (whether diabetic or not) who had the lowest glomerular filtration rate. The blood level of carbutamide showed poor correlation with response; carbutamide clearance was correlated with positive or negative response only in diabetics. There was a highly significant correlation between the urinary excretion of glucose and carbutamide. Reabsorption, degree of acetylation, and diuresis apparently had no relationship to the likelihood of response. It is concluded that the degree of glomerular elimination is important in determining the response to carbutamide administration but is not the only factor. W.R.K.

Jones, George M. (Dept. of Int. Med., Univ. of Texas Southwestern Medical School, Dallas, Tex.): POSTHYPOGLYCEMIC ENCEPHALOPATHY. South. M. J. 53:1258-62, October 1960.

The author surveys historically the development of knowledge concerning the damaging effect upon the brain of prolonged hypoglycemia, beginning with the first such case in an insulin-treated diabetic, reported in 1922 by Woodyatt. The author has designated this clinical entity as posthypoglycemic encephalopathy. He describes the various clinical manifestations resulting from it and their underlying pathology and points out the occasional concomitant adrenal necrosis and hemorrhage with increased sensitivity to insulin. The occurrence of the syndrome is cited in cases of idiopathic infantile hypoglycemia, mental disease given the Sakel form of insulin shock therapy, pancreatic adenoma, and diabetes under insulin treatment. The imperative treatment of an established hypoglycemic episode with adequate intravenous glucose is emphasized or, until this becomes available, with injections of epinephrine, or enemas of fruit sugar. For patients who fail to recover clinically after correction of hypoglycemia for a number of hours with intravenous glucose, the administration is suggested of steroids or ACTH for their anti-inflammatory effect. L.S.S.

Kalk, H. (City Hospital, Kassel, Germany): THE RELATIONSHIP BETWEEN FATTY LIVER AND DIABETES MELLITUS. German M. Month. 5:81-84, March 1960.

Liver biopsies in 121 diabetics revealed cirrhosis of the liver in thirty-nine. Even allowing for a special case selection, the coexistence of the two diseases must thus be much more frequent than has generally been assumed. In eighteen patients diabetes preceded cirrhosis, while in sixteen cirrhosis or hepatitis preceded the onset of diabetes. In the latter cases it is thought that hepatitis and the subsequent cirrhosis led to pancreatic disease, which also involved the islets. However, in the majority of cases diabetes is a primary disease. In a high percentage of cases of "senile" diabetes there is also fatty liver. The fatty liver of diabetes was characterized histologically by coarse fat droplets and prominent vacuolization, lighter color and variation in size of the liver-cell nuclei. This picture was also seen in those patients who did not develop diabetes until later, i.e., there exists a prediabetic stage which consists of a still compensated disorder of carbohydrate metabolism leading to fatty changes. W.R.K.

Kramer, David W. (Jefferson Med. College Hosp. and St. Luke's and Children's Med. Center, Philadelphia, Pa.): ATHEROMATOSIS—ITS RELATIONSHIP TO DIABETES MELLITUS. Nebraska M. J. 45:249-56, May 1960.

Previously, the term arteriosclerosis has been applied to practically all of the occlusive disorders except diabetic atheromatosis and thromboangiitis obliterans, as well as other forms of arterial thrombosis. This is not a desirable procedure because atheromatosis deserves a status as an entity among vascular disorders. It is basically and etiologically different from arteriosclerosis. An analysis of 3,800 diabetics, covering almost four decades, was arranged in series of 1,000 cases. They showed that the incidence of vascular disorders has been steadily rising from 17.3 per cent to 53.5 per cent. The question is posed as to whether it is justifiable to consider these developments as complications or are they really concomitants? The gradual and progressive increase of vascular disorders among diabetics is a challenge to the medical profession to make further investigations and to attempt to locate the more definite factors which produce atherogenesis and not to assume a state of complacency by attributing these vascular changes to the diabetes. W.R.K.

Leites, S. M.; and Smirnov, N. P. (All-Union Inst. of Experimental Endocrinology, Moscow, USSR): THE IMPORTANCE OF THE INSULIN INACTIVATING PROPERTIES OF THE LIVER (INSULINASE) IN THE MECHANISM OF ACTION OF ANTIDIABETIC SULFONAMIDE PREPARATIONS. Bull. Exper. Biol. & Med. (Russia) 47:711-14, June 1959.

Insulinase activity is either entirely absent or is considerably

lower in sexually immature rats weighing from 30 to 50 gm. Nadisan (BZ-55) has no hypoglycemic effect in the animals of this age. Insulinase appears with sexual maturity and then Nadisan exerts a hypoglycemic effect in these animals. Administration of chorionic gonadotropin or sex hormones to sexually immature rats is associated with appearance or intensification of insulinase activity and hypoglycemic effect of Nadisan. Injection of testosterone propionate to adult rats affects neither the hepatic insulinase activity nor the hypoglycemic effect of Nadisan. This shows that sex hormones have no direct activating action on insulinase but only promote the development of this enzyme system during ontogenesis. These data may mean that inactivation of hepatic insulinase by sulfonamides plays an important role in the mechanism of their hypoglycemic effect. W.R.K.

Lightbody, T. D.; and Reid, James (Clin. Chemotherapeutic Res. Unit of Med. Res. Council, Western Infirmary, Glasgow, Scotland): ORTHO-CRESOTINATE AND DIABETES MELLITUS. Brit. M. J. *1*:1704-07, June 4, 1960.

Ortho-cresotinate was tested in nine diabetic patients and had a hypoglycemic effect similar to aspirin. The undesirable side effects of deafness and tinnitus evident with aspirin were absent or minimal with this substance. J.A.

Lipsett, M. B.; Engel, Howard R.; and Bergenstal, D. M. (National Cancer Inst., National Institutes of Health, Bethesda, Md.): EFFECTS OF GLUCAGON ON PLASMA UNESTERIFIED FATTY ACIDS AND IN NITROGEN METABOLISM. J. Lab. & Clin. Med. 56:342-54, September 1960.

The injection of glucagon into subjects in a fasting state resulted in a rise in plasma unesterified fatty acids, four to six hours after injection. Glucagon administration also resulted in a negative nitrogen balance in five subjects which was shown to be independent of the adrenal pituitary axis. It is suggested that the catabolic effect of glucagon was not mediated by glycogenolysis and consequent gluconeogenesis, but it was proposed that glucagon increased the peripheral caloric demand, thereby leading to increased levels of unesterified fatty acids and the negative nitrogen balance. G.J.H.

MacHattie, Lorne A. (Dept. of Physiology, Univ. of Buffalo Sch. of Med., Buffalo, N.Y.): GRAPHIC VISUALIZATION OF THE RELATIONS OF METABOLIC FUELS: HEAT: O_2: CO_2: H_2O: URINE. N. J. Appl. Physiol. *15*:677-83, July 1960.

A system of graphing is presented whereby the relations of the various measured and calculated variables of metabolism can be shown and precisely described. Some consideration is given to unsteady as well as steady states. J.A.

Marks, Vincent (Dept. of Chemical Path., Inst. of Neurology, National Hosp., London, England): RESPONSE TO GLUCAGON BY SUBJECTS WITH HYPERINSULINISM FROM ISLET-CELL TUMOURS. Brit. M. J. *1*:1539-40, May 21, 1960.

The response to the intramuscular injection of 1 mg. of glucagon in four patients with hyperinsulinism is recorded. A hypoglycemic phase following a normal rise in blood sugar distinguishes it from the response observed in healthy subjects. The use of glucagon in the differential diagnosis of the hyperglycemic syndrome is discussed. W.R.K.

Martin, Maurice J.; Salassa, Robert M.; and Sprague, Randall G. (The Mayo Clinic, Rochester, Minn.): CLINICS ON ENDOCRINE AND METABOLIC DISEASES. 3. THE DEVELOPMENT OF DIABETES MELLITUS IN A PATIENT WITH PITUITARY INSUFFICIENCY AND RENAL GLYCOSURIA. Proc. Staff Meet. Mayo Clin. *35*:414-20, July 6, 1960.

In the reported case, the patient experienced the successive development of renal glycosuria, pituitary insufficiency due to postpartum pituitary necrosis (Sheehan's syndrome) and, finally, diabetes mellitus. This case differs from most other reported instances of the Houssay phenomenon in human beings in that pituitary insufficiency developed before diabetes and renal glycosuria were coexistent. W.R.K.

McIntosh, Hamish W.; Robertson, H. Rocke; Walters, Waltman; and Randall, Raymond V. (University of British Columbia, and Shaughnessy Hosp., Vancouver, B.C., Canada; Mayo Clinic and Mayo Foundation, Rochester, Minn.): FUNCTIONING ISLET-CELL CARCINOMA OF THE PANCREAS WITH METASTASES AND PROLONGED SURVIVAL. A.M.A. Arch. Surg. *80*: 1021-28, June 1960.

A fifty-one-year-old male lived fifteen years following removal of a malignant islet-cell tumor. Metastatic adenocarcinoma of the liver demonstrated at surgery ten years later was responsible for continued hypoglycemia due to insulin release. A.R.C., JR.

McMahon, F. Gilbert (Mahorner Clin. and Louisiana State University Med. Sch., New Orleans, La.): STEROID DIABETES. J. Louisiana M. Soc. *112*:126-30, April 1960.

By far the vast majority of patients receiving steroid therapy do not develop steroid diabetes. The incidence is less than 1 per cent. If the diabetic patient truly needs steroids, there should be no hesitation about their use. He may only require 15 to 20 units/day more of his insulin. The refractoriness of such patients to insulin is usually only relative. When the steroid therapy is withdrawn, of course, the steroid diabetes disappears within a few days. It is generally felt that a familial deficit in the potential reserves of the beta cells predisposes to the development of diabetes under sustained stress. The production of diabetes following pregnancy, burns, fractures, surgery, or the continued administration of large doses of steroids probably only occurs in the prediabetic subject. If one wishes to exclude the diabetes-susceptible patients before giving steroids, the cortisone glucose tolerance test of Conn might well be helpful. The treatment of steroid diabetes is either the administration of extra insulin, or the withdrawal of the steroid. W.R.K.

Mehnert, Hellmut; and Krall, Leo P. (Joslin Clinic, New England Deaconess Hosp., Boston, Mass., and Univ. Medical Policlinic, Munich, Germany): THE BIGUANIDE DERIVATIVES IN THE TREATMENT OF DIABETES MELLITUS. German M. Month. 5:225-28, July 1960.

Present-day knowledge of biguanides fails to explain their hypoglycemic effect, which is independent of pancreatic function and stimulates anaerobic glycolysis. The hypoglycemic effect is proportional to the dose administered. Concomitant gastrointestinal side effects associated with increasing dosage limit the use of biguanide therapy. Side effects disappear when dosage is lowered or the drug is withdrawn. Biguanides are particularly useful in combination with insulin in labile diabetics, when they produce a stabilizing effect, often lowering insulin dosage, and lessen the incidence of hypoglycemic "shock." Biguanides alone, or in combination with a sulfonylurea, may be successful in diabetics who are early or late sulfonylurea "failures." Patients who respond adequately to diet alone or sulfonylurea or insulin do not require biguanide therapy. A certain

amount of insulin, endogenous or exogenous, is essential for successful biguanide usage. W.R.K.

Mitchell, Marvin L. (Dept. of Med. and Radioisotope Unit, Lemuel Shattuck Hosp. and Tufts Univ. School of Med., Boston, Mass.): ABNORMAL INSULIN-BINDING FRACTION DEMONSTRATED BY THE ELECTROPHORESIS ON ION-EXCHANGE PAPER OF SERA FROM DIABETIC PATIENTS. J. Clin. Endocrinol. 20: 1319-32, October 1960.

A cation-exchange resin paper, Amberlite IR-120, was used as the supporting medium for the electrophoresis of serum. Insulin-I^{131}, alone, remained adherent to the origin of the resin paper, but migrated with a mobility that was almost as great as that of serum albumin when the insulin-binding sites of the paper had been saturated by stable insulin. Insulin-I^{131} in serum from normal subjects and from most of the insulin-responsive diabetic patients migrated with a mobility approximating that of alpha or beta globulins. However, insulin-I^{131} in serum from "true" insulin-resistant diabetic patients migrated in association with the gamma and inter-gamma-beta protein fractions. Thus, distinct physical differences were demonstrated between the serum insulin-binding proteins of insulin-responsive and insulin-resistant diabetic patients. W.R.K.

Nabarro, J. D. N. (Middlesex Hosp., London, England): THE PITUITARY AND ADRENAL CORTEX IN GENERAL MEDICINE. Brit. M. J. 2:553-58; 625-33, Aug. 20 and Aug. 27, 1960.

These essays were presented as the Oliver-Sharpey Lectures delivered before the Royal College of Physicians of London in March 1960. J.A.

Payne, R. W.; Diment, Dean H.; and Blaschke, John A. (Depts. of Pharmacology and Med., Univ. of Oklahoma School of Med., Oklahoma City, Okla.): EXTENDED TREATMENT OF DIABETES MELLITUS WITH CHLORPROPAMIDE (DIABINESE). J. Oklahoma M. A. 53:519-22, July 1960.

Seventeen diabetic patients treated with chlorpropamide are presented. Eleven of these patients (65 per cent) remain satisfactorily controlled on the drug in daily doses of 250-500 mg. after treatment periods of at least one year. Six of these patients were classified as secondary failures to tolbutamide therapy. No secondary failure of response to chlorpropamide has occurred in the present series. Delay in the onset of hypoglycemia occurred in two patients, requiring reduction in dosage of chlorpropamide. Symptoms of diabetic neuropathy were present in four of the cases at the beginning of chlorpropamide therapy and disappeared early during treatment with this agent. Side effects to chlorpropamide, generally manifested as gastrointestinal symptoms, developed in five patients one of whom developed hepatitis. The side effects observed were believed to be due to excessive dosage of the drug. Transient elevation of serum alkaline phosphatase was found in five patients of the present series. W.R.K.

ReMine, William H.; Scholz, Donald A.; and Priestley, James T. (Sections of Medicine and Surgery, Mayo Clinic and Mayo Foundation, Rochester, Minn.): HYPERINSULINISM: CLINICAL AND SURGICAL ASPECTS. Am. J. Surg. 99:413-19, April 1960.

Since the first case was described by Mayo in 1927, ninety-five functioning islet cell tumors have been discovered in 109 patients having hyperinsulinism at the Mayo Clinic. Eighty-five tumors were classified as adenomas, ten as carcinomas with metastasis. No cases of islet cell hyperplasia have been found to produce hyperinsulinism. Hypoglycemic symptoms are due to depression of oxidation in cells of the central nervous system. Incidence was greatest in the fourth to six decades of life. Positive family history for diabetes was established in twenty-three of eighty cases. Glucose tolerance tests were of little diagnostic value. Typical attacks of hypoglycemia could be reproduced in sixty-six patients. Functional hypoglycemia is a separate entity in which symptoms occur two to four hours after meals in patients having emotional difficulties. Surgical cure may be anticipated if a single benign adenoma is removed. The malignant tumors may be treated only temporarily with radiation or steroid therapy. A.R.C., JR.

Renold, Albert E.; Martin, Donald B.; Dagenais, Yves M.; Steinke, Jurgen; Nickerson, Rita V.; and Sheps, Mindel C. (Harvard Med. Sch.; the Peter Bent Brigham Hosp., and the Baker Clinic Research Lab., New England Deaconess Hosp., Boston, Mass.): MEASUREMENT OF SMALL QUANTITIES OF INSULIN-LIKE ACTIVITY USING RAT ADIPOSE TISSUE. I. A PROPOSED PROCEDURE. J. Clin. Invest. 39:1487-98, September 1960.

The authors describe the development of a method of measuring the presence of insulin-like activity by a biological system using the epididymal adipose tissue from the male albino rat of the Wistar strain.

The index used was the production of $C^{14}O_2$ by this tissue during incubation with glucose-1-C^{14} in the environment. The estimations were made by the use of dose response curves expressed as the logarithm of counts of radioactive carbon per minute per milligram of adipose tissue versus the added insulin or insulin-like material used for assay.

They assessed the influence of factors such as duration of inoculation time, ionic environment, concentration of glucose, addition of gelatin, the weight of the donor animals and the weight of the tissue fragments used.

This method is capable of detecting as little as 10 microunits of insulin per ml. and can be used to compare known and unknown insulin-like substances in a single day using the same animal's tissue.

Purified human albumin or gamma globulin had no effect. Glutathione had some effect in higher concentrations. Other hormonal substances likely to be found in blood would not affect the assay in the amounts ordinarily present. S.B.B.

Ries, W.; Schuster, L.; Seige, K.; and Wegner, H. (Dept. of Med. and the Isotope Lab. of the Dept. of Dentistry, Karl-Marx University, Leipzig, Germany): TISSUE-CLEARANCE WITH I^{131} IN DIABETES. Klin. Wchnschr. 38:681-83, July 15, 1960.

Into a circumscribed area of the gingiva 0.25 ml. physiological NaCl solution with 0.5 μc I^{131} was injected and the tissue clearance of I^{131} estimated with a scintillation counter. Similarly, clearance of the tissue in the intraclavicular area was determined after a subcutaneous injection of 0.25 μc I^{131}. The first series was carried out on 141 diabetic patients and thirty-two healthy control individuals, the second on eighty-one diabetics and 117 controls. In all diabetic patients the tissue clearance for I^{131} was decreased. The difference between the diabetics and the controls was significant (3δ:3.9). This difference was present in all age groups. The delay in I^{131} tissue clearance appeared to be directly related to the duration of the diabetes and to presence or absence of clinically apparent vascular complications. It was significantly greater in patients with

evidence of retinopathy than in those without retinopathy. The authors regard their results as further evidence of a universal abnormality of capillary functions in diabetes (German). M.G.G.

Sarcione, Edward J.; Sokal, Joseph E.; and Gerszi, Kornel E. (Div. of Med., Roswell Park Memorial Inst., Buffalo, N.Y.): RELATIONSHIP OF THE ADRENAL MEDULLA TO THE HYPERGLYCEMIC EFFECT OF GLUCAGON. Endocrinology 67:337-46, September 1960.

Glucagon failed to produce a rise in blood sugar when administered intravenously (0.4 mg./kg.) to adrenodemedullated rats. Adrenergic blockage produced by dihydroergotamine also prevented a response to glucagon. Administration of epinephrine re-established sensitivity to glucagon. Since both groups of animals had adequate initial stores of hepatic glycogen which fell after administration of glucagon it was concluded that the hyperglycemic effect of glucagon required epinephrine suppression of peripheral glucose uptake. H.L.W.

Scholz, Donald A.; ReMine, William H.; and Priestley, James T. (Rochester, Minn.): CLINICS ON ENDOCRINE AND METABOLIC DISEASES. 3. HYPERINSULINISM: REVIEW OF 95 CASES OF FUNCTIONING PANCREATIC ISLET CELL TUMORS. Proc. Staff Meet. Mayo Clin. 35:545-50, Sept. 14, 1960.

The clinical and pathologic findings in ninety-five proved functioning pancreatic islet cell tumors are reviewed. Eighty-five of the tumors have been classified as adenomas; this group includes twenty-four borderline tumors classified by the pathologists as showing grade-1 adenocarcinomatous changes. Ten tumors were carcinomas of islets with metastasis. Intolerance of patients with functioning islet cell tumors to prolonged fasting is a helpful and reliable diagnostic procedure in the detection of such tumors. In eighty-six of the patients the tumor was found at the first operation by the surgeon or in the resected pancreatic tissue by the pathologist, and a tumor subsequently was demonstrated in nine cases at reoperation or by the pathologist at necropsy. W.R.K.

Sheps, Mindel C.; Nickerson, Rita J.; Dagenais, Yves M.; Steinke, Jurgen; Martin, Donald B.; and Renold, Albert E. (Harvard Med. Sch. & Peter Bent Brigham Hosp.; and the Baker Clinic Research Lab., New England Deaconess Hosp., Boston, Mass.): MEASUREMENT OF SMALL QUANTITIES OF INSULIN-LIKE ACTIVITY USING RAT ADIPOSE TISSUE. II. EVALUATION OF PERFORMANCE. J. Clin. Invest. 39:1499-510, September 1960.

The authors describe the application of an assay method for insulin-like activity using rat epididymal adipose tissue suspended in glucose-1-C^{14}. This resulted in a linear dose response curve (expressed logarithmically) between $C^{14}O_2$ counts per minute and concentrations of standard insulin in a range of concentration from 31 to 500 micro-units per ml. The design of such a method for the routine assay of unknown amounts of insulin was described together with the details of the analysis of the results.

A definite increase in serum insulin activity occurred after intravenous infusion of glucose and reached a peak in twenty to thirty minutes; this did not occur after mannose. Moreover, the concentration of insulin was higher in pancreatic and portal veins than in the general circulation. It was found to be increased in the serum of patients with tumors of the pancreatic islets. All this supports the conclusion that insulin is being measured.

The increase in measurable insulin activity as a result of dilution of serum samples was much less than that observed using the rat diaphragm method.

However, the serum of recently pancreatectomized animals still contained a small though measurable amount of insulin-like activity in the fasting state. This discrepancy will require further investigation. S.B.B.

Shoemaker, William C.; Panico, Frederick G.; Walker, William F.; and Elwyn, David H. (Dept. of Exper. Surgery, Michael Reese Hosp., Chicago, Ill.): PERFUSION OF CANINE LIVER IN VIVO. J. Appl. Physiol. 15:687-90, July 1960.

A method is described for perfusing the canine liver in vivo and in situ with heparinized whole blood. Some preliminary data on concentration changes of several constituents of the perfusing fluid is presented. J.A.

Shoemaker, William C.; Teel, Peter (Dept. of Surg., Harvard Med. Sch., Boston, Mass., & Michael Reese Hosp., Chicago, Ill.): THE EFFECT OF GLUCAGON ON HINDQUARTER BLOOD FLOW IN THE DOG. Endocrinology 67:132-37, July 1960.

Intravenous administration of glucagon (0.02 mg./kg.) produced a decrease in hindquarter blood flow reaching a nadir in ten to twenty minutes. The fall in blood flow was simultaneous with a rise in hepatic blood flow suggesting a redistribution of cardiac output initiated by the drug. Administration of glucose or saline caused a transient increase in hindquarter flow. Fasting prevented the glucagon-induced vascular changes. H.L.W.

Smith, M. J. H.; and Moses, V. (Dept. of Chem. Pathology, King's College Hosp. Med. Sch., Denmark Hill, London, S.E. 5, England): UNCOUPLING REAGENTS AND METABOLISM. I. EFFECTS OF SALICYLATE AND 2:4-DINITROPHENOL ON THE INCORPORATION OF C^{14} FROM LABELED GLUCOSE AND ACETATE INTO THE SOLUBLE INTERMEDIATES OF ISOLATED RAT TISSUES. Biochem. J. 76:579-85, September 1960.

Studies on isolated rat tissues indicated salicylate and dinitrophenol considerably reduced the total incorporation of C^{14} from the labeled acetate, and the subsequent distribution of the isotope was restricted to the amino and organic acid fractions of the soluble intermediates.

The total incorporation and distribution of the C^{14} from the (C^{14})glucose were not materially altered in the presence of salicylate and dinitrophenol.

Intermediates of glycolysis HMS pathway, tricarboxylic acid cycle transamination and synthetic reaction have been separated by paper chromatography and counted for radioactivity. J.A.

Smith, M. J. H.; and Moses, V. (Dept. of Chem. Pathology, King's College Hosp. Med. Sch., Denmark Hill, London, S.E. 5, England): UNCOUPLING REAGENTS AND METABOLISM. II. EFFECTS OF 2:4-DINITROPHENOL AND SALICYLATE ON GLUCOSE METABOLISM IN BAKER'S YEAST. Biochem. J. 76: 585-94, September 1960.

The effects of salicylate and DNP on the metabolism of glucose UC^{14} in yeast was studied using paper chromatography. Intermediates of glycolysis, shunt, Krebs cycle and uridine diphosphate hexose metabolism, were isolated and counted for radioactivity.

Little difference was observed between the action of DNP and that of salicylate. J.A.

Stalder, G.; Schmid, R.; and Wolff, M. v. (Univ. of Basel, Switzerland): FUNCTIONAL MICRO-ANGIOPATHY IN DIABETIC CHILDREN. German M. Month. 5:113-16, April 1960.

Renal clearance values were determined in eighteen diabetic children four to eighteen years old requiring insulin. None of the patients had evidence of kidney disease and only one of retinopathy. It was found that within a few weeks of the manifestation of diabetes there occurred marked changes in renal function, which the authors have called functional renal micro-angiopathy. Contrary to findings in intercapillary glomerulosclerosis of the adult diabetic, the functional micro-angiopathies of the adult diabetic, the functional micro-angiopathy of the diabetic child is evidenced by an increase in insulin clearance and of the filtration fraction. It is suggested that the abnormal renal blood flow causes unphysiological variations and elevations of pressure in the glomerular capillaries; their increased permeability is the result of mechanical overstretching. Persisting or recurring functional micro-angiopathies may perhaps ultimately result in intercapillary glomerulosclerosis. W.R.K.

Steigerwald, H.; Spielmann, W.; Fries, H.; and Grebe, S. F. (Dept. of Med., the Transfusion Service, the Senckenberg Inst. of Pathology and the Radiological Inst., Univ. of Frankfort/Main, Germany): RECENT STUDIES ON THE ANTIGENICITY OF INSULIN. Klin. Wchnschr. 38:973-80, Oct. 1, 1960.

Rabbits were immunized with large doses of insulin, so that multiples of the lethal doses were tolerated. Crystalline, amorphous and cysteine-inactivated insulin were used. Active insulin was used in initial doses of 25 mg. = 600 U. I.V. or 5 mg. = 120 U. I.M., and administered twice weekly. The total doses were as high as 2 gm. within 150 days. Inactivated insulin was given in single doses of 80-100 mg. with a cumulative dose as high as 5 gm. in fourteen months. The rabbit immune-sera precipitated with purified Crystalline Insulin under formation of two lines of precipitates in both the Ouchterlony plate test and the immune-electrophoretic method of Grabar. These precipitated antibodies did not seem to bind the hormonally active group of the insulin-molecule. In other experiments an insulin-fluorescein product was used to demonstrate insulin antibodies in the sera of insulin resistant diabetic patients. This demonstration was successful in seventy-nine out of four hundred and sixteen such sera. The antibodies were found in the α_2 and γ_1 globulin fraction. In contrast to the studies with the rabbit immune-sera, these sera of the insulin resistant patients, as well as their α_2 and γ_1 globulin fractions were capable of inhibiting the hypoglycemic effect of insulin. Since, however, circulating antibodies were demonstrable in only a small group of insulin resistant diabetic patients, the authors proceeded to employ insulin-fluorescein to study the insulin distribution in normal and in immunized rabbits. They made the remarkable observation that in the latter animals the insulin-fluorescein was localized mainly in the lymph nodes and to a small degree in the bone marrow, whereas in the normal animals it was found almost exclusively in the liver. They concluded from this finding that insulin resistance may be caused not only by circulating antibodies but probably more often by sessile antibodies in the lymphatic tissue. In such instances the insulin may be retained by the lymph nodes and unable to reach the liver, which is the main place of its hormonal activity (German). M.G.G.

Stephens, John W.; Page, Otto C.; and Hare, Robert L. (2455 N.W. Marshall St., Portland 10, Oreg.): GALES CREEK CAMP—A SUMMER CAMP FOR DIABETIC CHILDREN. Northwest Med. 59:364-66, March 1960.

A progress report of Gales Creek Camp near Glenwood, Oregon, now in its fourth year of operation. The camp can accommodate twenty-five to thirty-five children between ages of eight to seventeen years. W.R.K.

Stolc, V.; and Pogády, J. (Endocrinological Inst. of the Slovak Academy of Sciences, Bratislava, and Psychiatric Dept., Pezinok, Czechoslovakia): THE EFFECT OF INSULIN AND CARDIAZOLE-SHOCK ON PROTEIN-BOUND IODINE IN BLOOD, STUDIED IN SCHIZOPHRENIC PATIENTS. Acta endocrinol. 34:157-62, May 1960.

Blood PBI levels decreased one-half hour after institution of insulin shock therapy in patients with acute but not with chronic schizophrenia. In both a subsequent rise, within the bounds of normal limits, occurred even in the face of glucose administration used to interrupt the insulin coma. After cardiazole, the shock resulted in a rise of the blood PBI within five minutes in both groups. The mechanism is as yet unexplained. S.B.B.

Sun, David C. H.; and Shay, Harry (Samuel S. Fels Res. Inst., Temple Univ. Med. Center, Philadelphia, Pa.): MECHANISM OF ACTION OF INSULIN HYPOGLYCEMIA ON GASTRIC SECRETION IN MAN. J. Appl. Physiol. 15:697-703, July 1960.

Results presented show a three component mechanism of action of insulin hypoglycemia on gastric secretion (1) an initial inhibitory effect on basal gastric secretion (2) a stimulating effect mediated through the vagus nerve (3) and a gastric secretory response mediated through the adrenal glands. J.A.

Tranquada, Robert E.; Solomon, David H.; Brown, Josiah; and Greene, Ruth (Dept. of Med., Univ. of Calif. Med. Sch., Los Angeles, Calif.): THE EFFECT OF ORAL HYPOGLYCEMIC AGENTS ON THYROID FUNCTION IN THE RAT. Endocrinology 67:293-97, September 1960.

Carbutamide was found to be goitrogenic in addition to reducing I^{131} uptake and I^{127} concentration in the thyroid of the rat. Metahexamide showed a less striking effect on thyroid iodine uptake and concentration. Tolbutamide, chlorpropamide, and phenethylbiguanide did not interfere with thyroid activity. H.L.W.

Volk, Bruno W. (Jewish Chronic Disease Hosp., Brooklyn, N.Y.): SPONTANEOUS HYPOGLYCEMIA WITH ABDOMINAL SPINDLE-CELL SARCOMA. Geriatrics 15:473-79, June 1960.

This is a report of clinical pathologic conference concerning a seventy-year-old man with repeated hypoglycemic attacks in association with a large sarcoma of the small intestine. Postmortem examination failed to disclose any significant abnormalities of the pancreas. Assays of the blood and tumor tissue for insulin-like activity were carried out. No significant activity was found in the blood. However, an activity of 1.8 units per kilogram could be demonstrated in tumor tissue which would equal approximately 20 units for the entire growth. This c illustrates a relatively rare cause for hypoglycemia. Most of the tumors have been fibrogenic sarcomas as in this case and were usually located in the abdominal cavity. S.S.

ORGANIZATION SECTION

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21st ANNUAL MEETING

The American Diabetes Association will hold its Twenty-first Annual Meeting at the Hotel Commodore in New York City, June 24-25, 1961, immediately prior to the annual session of the American Medical Association. Pertinent information about the Annual Meeting, together with a hotel reservation form, was mailed to members of the Association in November. *Those members who plan to attend the meeting should complete the form and send it directly to the Hotel Commodore as soon as possible.*

The Annual Banquet and Social Hour will be held Saturday evening, June 24. Members, their wives (or husbands) and friends are cordially invited to attend both the Banquet and Social Hour. A reservation form for the Banquet will be mailed a few months prior to the meeting.

If you are planning to attend the ADA Twenty-first Annual Meeting and to remain for the annual meeting of the American Medical Association which follows on June 26-30, be sure to inform the Hotel Commodore that you would like to continue your reservation for the AMA sessions.

SCIENTIFIC PROGRAM

Rachmiel Levine, M.D., Chairman and Professor, Department of Medicine, New York Medical College; Director, Medical Services, Flower and Metropolitan Hospitals, New York City, will deliver the Banting Memorial Lecture.

Physicians and other scientists who would like to present papers at the Scientific Sessions are invited to submit abstracts to Jerome W. Conn, M.D., Chairman of the Committee on Scientific Programs. These sessions will be held on Saturday and Sunday, June 24-25. Seven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent as soon as possible, but not later than March 1, 1961, to Dr. Conn in care of the national office.

A list of papers to be given at the Scientific Sessions will be published in DIABETES, and the program itself, including abstracts of all the papers, will be mailed to members before the meeting.

* * *

As announced in the November-December Journal, the Twenty-second Annual Meeting will be held June 9-10, 1962, at the Conrad Hilton Hotel, Chicago.

4th CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

The Fourth Congress of the International Diabetes Federation will be held in Geneva, Switzerland, July 10-14, 1961. Prof. Dr. J. P. Hoet, Louvain, is President of the Federation, whose other officers are: *Honorary Presidents*: E. P. Joslin, M.D., Boston; Charles H. Best, C.B.E., M.D., Toronto; R. D. Lawrence, M.D., London; and B. A. Houssay, M.D., Buenos Aires. *Vice Presidents*: Prof. Dr. K. Oberdisse, Düsseldorf; Prof. Dr. P. Rambert, Paris; Howard F. Root, M.D., Boston; Dr. F. G. Schlesinger, Utrecht, Pays-Bas; Dr. M. Silvestri-Lapenna, Rome; Mr. M. V. Steenberg, Copenhagen; and Madame G. Vernet, Geneva; *Acting Secretary*: Mr. James G. L. Jackson, London.

Officers of the Congress include: *President*: Prof. Dr. Hoet, Louvain; *President of the Organizing Committee*: Eric Martin,

M.D., Geneva; *Vice Presidents*: Georg Constam, M.D., Zurich, and Madame G. Vernet, Geneva; *Treasurer*: Mr. Philibert Lacroix, Geneva; *Secretary General*: Bernard Rilliet, M.D., Geneva.

All correspondence relating to the Congress should be addressed to Dr. Bernard Rilliet, 4, Boulevard des Tranchees, Geneva, Switzerland. March 31, 1961, is the closing date for registration. Application to read a paper or to show a film must be received not later than Feb. 1, 1961.

The American Diabetes Association Advisory Committee for the program includes Howard F. Root, M.D., Boston, Chairman; Alexander Marble, M.D., Boston; and Franklin B. Peck, Sr., M.D., Indianapolis. Those interested in group transportation should communicate as soon as possible with Howard F. Root, M.D., 15 Joslin Road, Boston 15, Mass. Round-trip fare by chartered plane is available at approximately $300. The plane will leave New York in the late morning of Saturday, July 8, 1961, will stop at Boston, and will return from Geneva four weeks later.

The preliminary program for the Congress follows:

GENERAL PROGRAM
Medical and Scientific Section

(NOTE: The official Congress languages will be French, German and English. There will be simultaneous interpretation in these languages.)

Monday afternoon, July 10, 1961.

Opening Ceremony. Addresses by official representatives, by the President of the Congress and by the President of the Organizing Committee.

"Experimental Diabetes and Human Diabetes," by Charles H. Best, Toronto, Canada.

"Physiopathology of Diabetes Mellitus," by B. Houssay, Buenos Aires; A. E. Renold, Boston; and C. N. H. Long, New Haven.

Tuesday morning, July 11, 1961.

"Diabetes in the Adolescent," by Priscilla White, Boston; P. Royer, Paris; and Y. Larsson, Stockholm. Discussion by C. Faelli, Rome; H. Krainick, Freiburg im Breisgau; and Madame M. L. Saldun de Rodriges, Montevideo.

Tuesday afternoon, July 11, 1961.

Specialized Clinics for Diabetic Patients

"Organization, Results, Experience," by Frau H. Bernard, Berlin; and K. Lundbaek, Aarhus.

"Medico-Social Aspects," by P. Petrides, Duisburg.

Wednesday morning, July 12, 1961.

"Prediabetic Conditions and Early Detection of Diabetes," by J. W. Conn and S. S. Fajans, Ann Arbor; P. Rambert, Paris; V. Schliack, Berlin; and W. P. U. Jackson, Cape Town. Discussion by P. A. Bastenie, Bruxelles; K. Oberdisse, Düsseldorf; R. Unger, Dallas; and H. Kalk, Kassel.

Thursday morning, July 13, 1961.

Neurological and Bone Complications in the Diabetic Patient.

"Bone Complications," by E. Azerad, Paris. Discussion by J. Mirouze, Montpellier; and Schmitt-Rohde, Berlin.

"Neurological Complications," by S. Fagerberg, Goteborg; M. Ellenberg, New York; and M. Derot and Morel-Maroger, Paris.

Thursday afternoon, July 13, 1961.

Oral Treatment of Diabetes.

"Sulfonylureas," by E. F. Pfeiffer, Frankfurt. Discussion by J. M. Stowers, Dundee; F. Bertram, Hamburg; S. S. Fajans, Ann Arbor; W. Meier, Frankfurt; A. L. Loubatieres, Montpellier; and W. Creutzfeldt, Freiburg im Breisgau.

"Biguanides," by M. Mehnert, Munich. Discussion by G. Ungar, New York; J. Sterne, Paris; R. Tranquada, Los Angeles; L. P. Krall, Boston; and A. Bloom, London.

Friday morning, July 14, 1961.

"Brittle Diabetes," by J. J. Groen, Jerusalem; A. Marble, Boston; and E. Rossi, Bern. Discussion by M. Somogyi, St. Louis.

Panel Discussions

Tuesday morning, July 11, 1961.

"Action of Insulin," Chairman, R. Levine, New York.

Wednesday morning, July 12, 1961.

"Lipid Metabolism in Diabetes Mellitus," Chairman, A. E. Renold, Boston.

Thursday morning, July 13, 1961.

"The Diabetic Coma," Chairman, H. C. Plattner, Geneva.

"Diabetes During Gestation and Its Influence on Fetal Pathology and Neonatal Behavior," Chairman, J. P. Hoet, Louvain.

Friday morning, July 14, 1961.

"Lasting Consequences of Hypoglycemia," Chairman, G. Mohnike, Berlin.

Section for Medical and Social Problems

Monday afternoon, July 10, 1961.

Opening Ceremony.

Tuesday morning, July 11, 1961.

"Psychology of the Young Disabled."

"The Diabetic Child, Family Environment, and the Physician," by Madame A. Margolis, Lodz.

Tuesday afternoon, July 11, 1961.

"The Diabetic Child in the School," by H. Krainick, Freiburg im Breisgau.

"Holidays and Leisure for Diabetic Children," by H. Lestradet, Paris.

"Medico-Social Aspects in the Organization of Diabetes Clinics," (common meeting with the scientific section) by P. Petrides, Duisburg.

Wednesday morning, July 12, 1961.

"Problems of the Aged and Disabled Diabetic," by H. F. Root, Boston.

"Institutions for the Aged and Disabled Diabetic," by Miss I. H. Rogers, London.

Thursday morning, July 13, 1961.

"Prospects of the Diabetic Adolescent in Private Life," by A. Mirsky, Pittsburgh.

"School and Career Problems of the Diabetic Adolescent," by Madame M. Parmentier, Paris; and Mademoiselle G. Bleger, Strasbourg.

Thursday afternoon, July 13, 1961.

"Special Problems Confronting the Doctor Treating the Diabetic Child," by R. Deuil, Paris; and Madame Hulot, Paris.

"Diabetes and Sports," by C. Benelli, Paris.

Friday morning, July 14, 1961.

"The Medico-Social Team and the Diabetic Adult," forum organized by B. Curchod, Lausanne.

Friday afternoon, July 14, 1961.

"Occupational Problems of the Diabetic Adult," by Mademoiselle E. H. J. Wijnmalen, Leiden.

"Public Information by Diabetes Associations," by J. G. L. Jackson, London.

MARCH 15 DEADLINE FOR ADA ESSAY CONTESTS

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage medical students, interns, and physicians within two years after their graduation from medical school, and graduate students in the basic sciences to enter the 1960-61 Graduate and Medical Student-Intern Essay Contests. All manuscripts should be typewritten and double-spaced, and mailed with a letter of transmittal by March 15, 1961, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y.

$250 Prize—for the best paper reporting original work, whether laboratory investigation or clinical observation.

$100 Prize—for the best review article or case report.

The prize-winners as well as those receiving honorable mention also are given a one-year's subscription to DIABETES: *The Journal of the American Diabetes Association.*

The papers will be judged on the basis of the value of the material and method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Papers entered in the Contests should not have been previously published. Appropriate manuscripts will be considered for publication in DIABETES.

Contestants should submit the original and two copies of their papers.

SPECIAL SUBSCRIPTION RATES

Medical Students, Interns and Residents may subscribe to DIABETES at a special rate of $4.50 per year, half of the regular subscription rate.

DIABETES WEEK 1960

With the announcement of an increase to 1,250,000 in the number of unknown diabetics in the United States, Franklin B. Peck, Sr., M.D., Indianapolis, President of the American Diabetes Association, launched what proved to be a highly successful Diabetes Week on Nov. 13, 1960, both in number of persons tested and in dissemination of educational materials.

A telegram from Augusta, Georgia, was a highlight of the week. In it, President Dwight D. Eisenhower commended the American Diabetes Association for "performing a fine public service in its program detecting signs of diabetes among our people." He also stated that "The Association's efforts have led to the early discovery of this illness in many citizens. By offering them an opportunity for early diagnosis, it provides hope for successful treatment."

MEAL PLANNING PUBLICATIONS

Meal Planning with Exchange Lists, a twenty-four-page booklet prepared to help diabetics select foods for their meals, is available at 15¢ each; $6.50 per 100 copies and $50.00 per 1,000 copies. Meal Plans No. 1 through No. 9 (1200, 1500, 1800, 2200, 1800 (includes more milk than No. 3), 2600, 3500, 2600 (includes less milk than No. 6), and 3000 calories respectively); "ADA Bland Low-fiber Diabetic Diet"; and "ADA Sodium Restricted Diabetic Diet," to be used in conjunction with the *Meal Planning* booklet, as well as the "Diabetic Diet Card for Physicians," may be secured at 5¢ per single copy; $2.00 per 100 copies; $18.00 per 1,000 copies. All costs include handling and shipping.

Order forms for the above material are available on request from the American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y.

DIABETES BINDERS AVAILABLE

Sturdy binders for your 1961 issues of DIABETES are now available at $2.25 each. Binders for prior years also are available at the same price.

A full set of binders for all ten volumes (1952-1961 issues) is offered at a package price of $20.00.

Each of the attractive binders will hold a year's issues of DIABETES and the annual index supplement. Of simulated dark blue leather, they will grace your library shelf and add years of life to your copies of DIABETES. Address your orders now for immediate shipment to: American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y.

"ARE DIABETICS GOOD LIFE INSURANCE RISKS?" IN ADA *FORECAST*

Studies of diabetic persons insured during the past two decades are the basis of a timely article which was published in the September-October 1960 issue of FORECAST. J. T. Sheridan, M.D., is the author. Physicians and others may wish to call this article to the attention of their diabetic patients and friends who may be concerned with questions related to their insurability. Copies of the September-October issue are 35¢ each or six for $2.00.

NEW MEMBERS

The following were elected as of Dec. 1, 1960, and Jan. 1, 1961:

Active

Florida	
Berry, James F.	Ft. Lauderdale
Kentucky	
Billington, C. B.	Paducah
Coleman, F. D.	Louisville
Michigan	
Moore, P. J.	Owosso
Vecchio, Thomas J.	Kalamazoo
Minnesota	
Ivy, Horace K.	Rochester
Purnell, Don C.	Rochester
Weeks, Richard E.	Rochester

New Jersey	
Henneman, D. H.	Jersey City
New York	
Avlonitis, E. G.	Buffalo
Shapiro, Norton	Niagara Falls
Oklahoma	
Hennes, A. R.	Oklahoma City
Pennsylvania	
Greenberg, S. R.	Abington
Wisconsin	
Heersma, J. R.	Marshfield
Other Countries	
Mexico	
Garcia-Reyes, Jose A.	Mexico City

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION held its annual symposium on Nov. 11, 1960, at Passavant Memorial Hospital. Prof. W. H. J. Butterfield delivered the Fourth Rollin T. Woodyatt Memorial Lecture, titled "New Thoughts on an Old Disease." Prof. Butterfield is Chairman of the Department of Experimental Medicine at Guy's Hospital Medical School, London. Peter Wright, M.D., also of Guy's, presented a paper entitled "Insulin Antagonists." He is continuing research in diabetes in the laboratories of the Department of Medicine at Northwestern University Medical School on a Rockefeller Foundation Fellowship. This lecture is made possible by a gift to Northwestern University by an anonymous devoted friend and admirer of the late Dr. Woodyatt. The first three Woodyatt Memorial Lectures were given by the late Prof. Evarts Graham of St. Louis, and Profs. Charles H. Best, Toronto, and Francis D. W. Lukens, Philadelphia.

NEWS NOTES

NEW FDA HEADQUARTERS IN 1963

The Food and Drug Administration's Washington operations will be consolidated in a modern $12,212,800 building scheduled to be completed in March, 1963. Location of the building in Southwest Washington is an expansion of the Department of Health, Education and Welfare headquarters there.

PHILADELPHIA LECTURE SERIES

A series of lectures on clinical nutrition for the house and medical staff of the Philadelphia General Hospital have been arranged by the Committee on Nutrition and Metabolism of the Philadelphia County Medical Society jointly with the National Vitamin Foundation and the Department of Education of the hospital.

The lectures will be held monthly in the Mills Auditorium at 8:00 p.m. on the following dates: *Jan. 18, 1961*—Richard W. Vilter, M.D., Cincinnati, "Nutrition in Relation to Heart Disease"; *February 15*—Harold A. Zintel, M.D., New York City, "Fluid Balance"; *March 15*—Edward H. Reisner, Jr., M.D., New York City, "Vitamin B-12 and Folic Acid"; *April 19*—Maurice E. Shils, M.D., New York City, "Metabolic and Nutritional Aspects of Renal Disease"; *May 10*—Herbert Pollack, M.D., New York City, "Malnutrition"; and *June 7*—Paul Gyorgy, M.D., Philadelphia, "Nutrition and Liver Disease."

ONE-DAY SYMPOSIUM

"Mechanisms of Gastrointestinal Absorption" is the title of a one-day symposium which will be held at the Hotel Sheraton-East, New York City, beginning at 9 a.m., March 7, 1961. The symposium will be presented under the joint sponsorship of The Johns Hopkins University, School of Hygiene and Public Health, and The National Vitamin Foundation. Advance registration is requested. There is no registration fee.

BRITISH DIABETIC ASSOCIATION TO MEET

The spring meeting of the Medical and Scientific Section of The British Diabetic Association will be held at University College Hospital, London, on April 7 and 8, 1961. Any member of the American Diabetes Association is cordially invited to attend. Write the Secretary of the Section at 152 Harley Street, London, W. 1.

NEW CHEMOTHERAPY JOURNAL

Chemotherapia, International Journal of Pharmacology, Toxicology, Clinic and Therapy, and official organ of the International Symposia of Chemotherapy in Geneva, has begun publication in Basel, Switzerland. Copies of the journal will appear every two months. Editors-in-chief of the publication are Drs. H. P. Kuemmerle, Tubingen, and P. Rentchnick, Geneva.

ANNUAL MEETING OF THE AMERICAN DIETETIC ASSOCIATION

The American Dietetic Association held its 43rd Annual Meeting in Cleveland, Ohio, from October 18 through 21. The four-day meeting took place at the Public Auditorium and the Sheraton-Cleveland, the headquarters hotel.

Authorities representing the fields of nutrition, diet therapy, food service administration, food technology, education, medicine and biochemistry presented papers, and more than 180 exhibitors participated in an exhibition at the Public Auditorium.

THE MARY PUTNAM JACOBI FELLOWSHIP

Each year the Women's Medical Association of the City of New York offers the Mary Putnam Jacobi Fellowship to a graduate woman physician, either American or foreign, for medical research, clinical investigation or postgraduate study in a special field of medicine. The Fellowship, which consists of $1,000, will begin Oct. 1, 1961, for a period of one year. At the discretion of the Committee, an award of $2,000 may be given biannually.

The recipient of the Fellowship will be expected to make a report at the end of the fourth month following which the balance will be awarded, subject to the approval of the Com-

mittee. The recipient is expected to devote full time to the Fellowship, but exceptions may be made under special circumstances. Applications may be obtained from the Chairman, Ada Chree Reid, M.D., 118 Riverside Drive, New York 24, N.Y., and must be returned before March 1, 1961. Successful candidates will be notified not later than May 1, 1961.

PERSONALS

ADA MEMBERS TAKE PART IN POSTGRADUATE COURSES

The following members of the American Diabetes Association will take part in these Postgraduate Courses to be offered by The American College of Physicians:

Officers of Instruction for Postgraduate Course No. 5, "Mechanisms of Disease," to be given at Columbia-Presbyterian Medical Center, New York, Jan. 16-20, 1961, include:

PHILIP H. HENNEMAN, M.D., Associate Professor of Medicine, Seton Hall School of Medicine, Jersey City, New Jersey;

ABBIE I. KNOWLTON, M.D., Assistant Professor of Medicine;

THEODORE B. VAN ITALLIE, M.D., A.C.P. Associate, Associate Clinical Professor of Medicine.

Officers of Instruction for Postgraduate Course No. 6, "Clinical Patho-Physiologic Conferences," to be given Feb. 20-24, 1961, at Oklahoma University Medical Center, Oklahoma City, Oklahoma, include:

R. PALMER HOWARD, M.D., Associate Professor of Research Medicine; Associate Head, Cardiovascular Section, Oklahoma Medical Research Foundation;

BERT F. KELTZ, M.D., Clinical Professor of Medicine; A.C.P. Governor for Oklahoma;

ROBERT LAWSON, M.D., ADA Governor for Oklahoma, Associate Professor of Medicine;

KELLY M. WEST M.D., Assistant Professor of Medicine; Chief, Diabetes Section, V.A. Hospital.

The following members of the American Diabetes Association have taken part in Postgraduate Courses already presented:

ROBERT H. WILLIAMS, M.D., Seattle, was director of Postgraduate Course No. 4 of The American College of Physicians. Entitled "Recent Advances in Drug Therapy," the course was given January 9-13, at the University of Washington School of Medicine in Seattle.

WILLIAM F. BRADLEY, M.D., Columbus, Ohio, was a member of The Ohio State University College of Medicine Faculty which presented Postgraduate Course No. 1 Sept. 19-24, 1960, at The Ohio State University Health Center. The Course was entitled "The Place of Hematology in Internal Medicine: with an Introduction to Radioisotope Technics and Their Application."

SOLOMON A. BERSON, M.D., and ROSALYN S. YALOW, PH.D., both of the Bronx Veterans Administration Hospital, have been named recipients of the first William S. Middleton Medical Research Award for their studies on insulin-binding antibodies and the immuno-assay of plasma insulin. The award has been established to honor the Veterans Administration's Chief Medical Director, who is a former Dean and Professor of Medicine of the University of Wisconsin Medical School.

JEROME W. CONN, M.D., Ann Arbor, Michigan, has been selected as Henry Russel Lecturer at the University of Michigan. The Russel Lecture is considered the University's highest professional recognition of academic and research competence. Dr. Conn is Professor of Medicine and Director of the Division of Endocrinology and Metabolism and the Metabolic Research Unit at the University of Michigan Medical Center. The author of more than 150 original scientific publications and articles, and Vice President of the American Diabetes Association, he will deliver the Russel Lecture this spring.

HAROLD RIFKIN, M.D., New York, is an Officer of Instruction in the Medical Division of the Postgraduate Courses in Clinical Medicine given in affiliation with Columbia University at Montefiore Hospital for the September 1960-June 1961 academic year.

CHARLES R. SHUMAN, M.D., Associate Professor of Medicine, Temple University School of Medicine, delivered a paper on "Surgery for the Diabetic Patient," on Oct. 28, 1960, in Dayton, Ohio, at a meeting of the Society of Internal Medicine. He also delivered papers on November 16 at the Kansas University School of Medicine in Kansas City on "Use and Abuse of Newer Nutritional Knowledge," and "Nutritional Aspects of Congestive Failure."

MAXWELL SPRING, M.D., New York, was Chairman of the "Internist's Day Program" sponsored Nov. 9, 1960, by The Bronx Chapter of the New York State Society of Internal Medicine.

THEODORE B. VAN ITALLIE, M.D., New York, Associate Clinical Professor of Medicine, and Chief of Medical Service, St. Luke's Hospital, is an Officer of Instruction at the Institute of Nutrition Sciences 1960-61, Columbia University, New York City. The academic year began Sept. 15, 1960, and ends May 27, 1961.

NECROLOGY

LOUIS B. BALDWIN, Phoenix, Arizona, born December 3, 1890.

KURT B. BLATT, Haverstraw, New York, born October 15, 1903.

HAROLD S. DAVIDSON, Atlantic City, New Jersey, born April 27, 1892.

OSCAR JULIUS EICHHORN, Pittsburgh, Pennsylvania, born June 29, 1894.

HARVEY L. FULLER, Baltimore, Maryland, born March 10, 1914.

CHARLES G. JOHNSTON, Detroit, Michigan, born March 13, 1899.

SAMUEL H. SCHWARTZ, Plainfield, New Jersey, born August 7, 1902.